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SINGLE NUCLEOTIDE POLYMORPHISMS IN INSULIN-LIKE GROWTH FACTOR (IGF) GENES AND THEIR ASSOCIATIONS WITH GROWTH IN LOCAL GUINEA FOWLS *(NUMIDA MELEAGRIS)* OF GHANA

BY

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DECLARATION

I, Kurukulasuriya Mariesta Jayaroshini Ahiagbe declare that the content of this thesis is result of my own research, field and laboratory work. This thesis has not been presented for the award of any degree at any institution anywhere in the world.

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ABSTRACT

Due to the pivotal role of Insulin-like Growth Factor 1 (IGF1) and Insulin-like Growth Factor 2 (IGF2) in growth regulation of poultry, the IGF1 gene (*gIGF1*) and IGF2 gene (*gIGF2*) in guinea fowl were examined as candidate genes for early growth in helmeted guinea fowls (*Numida meleagris*) from three populations of Northern Ghana (TPNG). Keets hatched from eggs collected from 32 sample locations comprising 11 subpopulations across three main populations located in Upper East Region, Upper West Region, Northern Region, North East Region, Savannah Region and an experimental flock maintained at Animal Research Institute (ARI) were raised and appraised for body weight and growth rate traits up to 11 weeks. Protein coding exons of *gIGF1* and selected targets of *gIGF2* were sequenced, aligned for discovery of novel Single Nucleotide Polymorphisms (SNPs) and genotyping. Effect of the genotypes at each SNP and *gIGF1* haplogroups were estimated using linear models. Birds from the TPNG did not vary in body weights and weekly growth rates among the populations and with that of ARI flock from the fourth week ($p > 0.05$). However, birds from subpopulations within the three main populations varied significantly ($p < 0.05$) in weekly body weights and growth rates from the second week, with between subpopulation variations becoming pronounced after the sixth week. Although ARI flock did not vary with other three populations in terms of body weight and growth rate, they demonstrated remarkably high survivability. In total six novel SNPs were identified within *gIGF1* including two SNPs within 5' Untranslated Region (UTR), one SNP in the second protein coding exon and three SNPs within 3'UTR. These SNPs were distributed among seven haplotypes and eight haplogroups among local guinea fowls from Northern Ghana. SNPs within the 5'UTR and 3'UTR had significant effects on body

weights and weekly growth rates from the second and fourth week, respectively, indicating possible roles for these polymorphisms influencing IGF1 synthesis at the translation level, and need to be further investigated to decipher the underlying molecular mechanisms. The only synonymous SNP located at the second protein coding region in *gIGF1* and both SNPs identified in *gIGF2* did not influence early growth in local guinea fowls from TPNG. The study provides baseline information on novel SNPs in *gIGF1* and their associations with body weight traits and early growth rates in local guinea fowls from Northern Ghana. It is recommended that the effect of the SNPs residing within 5'UTR and 3'UTR in *gIGF1* should be further investigated up to slaughter stage in structured breeding programmes aimed at developing fast growing guinea fowl breeds with pedigree data to facilitate their use in Marker Assisted Selection.

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DEDICATION

I dedicate this endeavour to

My God and Father

for immense love, direction and support,

My family

for their love and care,

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ACRONYMS AND ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphic DNA
AGDP	Agricultural Gross Domestic Product
AnGR	Animal Genetic Resources
ALBC	American Livestock Breed Conservancy
CSIR-ARI	Animal Research Institute of the Council for Scientific and Industrial Research
EBV	Estimated breeding values
ESTs	Expressed Sequence Tags
FAO	Food and Agriculture Organization of the United Nations
FAO-STAT	Statistical Databases of Food and Agriculture Organization of the United Nations
GEBV	Genome Enhanced Breeding Value
GS	Genomic Selection
GSS	Ghana Statistical Service
GWAS	Genome Wide Association Studies
GH	Growth Hormone
GHR	Growth Hormone Receptor
GHRH	Growth Hormone Releasing Hormone
HLGF	Helmeted guinea fowl (*Numida meleagris*)
IGFs	Insulin-like Growth Factors
IGF1	Insulin-like Growth Factor 1
IGF1	Insulin-like Growth Factor 1 gene

cIGF1	Insulin-like Growth Factor 1 protein in chicken *(Gallus gallus*)
cIGF1	Insulin-like Growth Factor 1 gene in chicken *(Gallus gallus*)
gIGF1	Insulin-like Growth Factor 1 protein in guinea fowl (*Numida meleagris*)
gIGF1	Insulin-like Growth Factor 1 gene in guinea fowl (*Numida meleagris*)
hIGF1	Insulin-like Growth Factor 1 protein in Humans
IGF2	Insulin-like Growth Factor 2
IGF2	Insulin-like Growth Factor 2 gene
cIGF2	Insulin-like Growth Factor 2 protein in chicken (*Gallus gallus*)
cIGF2	Insulin-like Growth Factor 2 gene in chicken (*Gallus gallus*)
gIGF2	Insulin-like Growth Factor 2 protein in guinea fowl *(Numida meleagris)*
gIGF2	Insulin-like Growth Factor 2 gene in guinea fowl *(Numida meleagris)*
IGFBPs	Insulin-like Growth Factor Binding Proteins
IGF1R	Insulin-like Growth Factor 1 Receptor
IGF1R	Insulin-like Growth Factor 1 Receptor gene
LD	Linkage Disequilibrium
MAS	Marker Assisted Selection
MOH	Ministry of Health, Republic of Ghana
NCBI	National Centre for Biotechnological Information, United States National Library of Medicine, National Institute of Health, Maryland, USA
NGS	Next Generation Sequencing
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Loci
RDAs	Recommended Dietary Allowances

RAPDs	Random Amplified DNA markers
RFLPs	Restriction Fragment Length Polymorphisms
SNP	Single Nucleotide Polymorphism
TPNG	Three guinea fowl populations of Northern Ghana from Upper East Region, former Northern Region and Upper West Region
UN	United Nations
UN Comtrade	United Nations Commodity Trade Statistics Database
5'UTR	5' Untranslated region
3'UTR	3' Untranslated region
WGS	Whole Genome Sequencing

CHAPTER 1

GENERAL INTRODUCTION

1.1. Background

Proteins are important biopolymers made up of amino acid monomers in all living organisms. By far, proteins are the most versatile biomolecules with diverse functions from cellular organization to modulating most complex biological functions such as human intelligence and immunity. At cellular level, they are important structural constituents of membranes and organelles. They also mediate intermediary metabolism by acting as receptors, enzymes and transporters. Highly specialized tissues in humans such as muscles, brain and skeleton are largely made up of proteins. At systemic level they play important roles as hormones and immunoglobulins, with no biological function possible without proteins (Buxbaum, 2015).

All proteins of the human body are produced from endogenous or dietary amino acids. A group of amino acids referred to as essential amino acids cannot be synthesized by the body and can only be supplied by the diet. Therefore, inadequate dietary protein, rich in essential amino acids, has negative effects on tissue development, cellular and physiological functions from conception to adulthood. Adequate but moderate level of proteins is also important in management of emerging non communicable conditions like diabetes and obesity. Therefore, a healthy human needs an optimum age dependent level of quality proteins for growth, maintenance, immunity and for optimal mental and physical functions (Nelson and Cox, 2005).

Food from animal sources are rich sources of essential amino acids and micronutrients with a higher bioavailability compared to plant based protein sources (Gibson, 1994). A diet deficient in animal source proteins, due to low income or due to spiritual believes in

affluent countries results in stunted growth, decreased cognitive development and decreased immunity during childhood (Dagnelie *et al.*, 1991; Neumann *et al*., 2002). Although the overall protein requirement reduces from childhood to adulthood, a healthy adult needs an adequate quantity of quality proteins from diverse sources. In the recent past, association of high intake of red meat from beef, chevron, mutton or pork with greater risk of cardiovascular heart disease and colon cancer has been suggested and debated (McAfee *et al*., 2010). Epidemiological studies conducted across the globe suggest lean poultry meat as the healthiest meat options available (Marangoni *et al*., 2015).

According to dietary and physical activity guidelines prescribed by the Ministry of Health of Ghana, both men and women need an average of 0.8 gram of protein/kilogram of body weight while children, pregnant and lactating mothers have greater protein requirements. Therefore, the ranges for Recommended Dietary Allowances (RDAs) for protein are 16-28, 45-63, and 46-50 g for children, adult males and adult females, respectively, while for pregnant and lactating mothers the RDAs are 60 g and 62 g respectively (MOH, 2010). Over the past decades, the country has gained significant improvements, reaching an average per capita protein consumption of 59g /person/day at national level (FAO-STAT, 2010).

Although the average national protein intake has demonstrated modest gains over the years, reaching the recommended levels, an extensive review of trends of per capita protein intake by regions, age groups and the quality of proteins, points out several disparities in quantities and quality of protein consumed by the Ghanaian populace. Aside an encouraging per capita protein intake on a quantitative scale, the quality of protein consumed by vast majority of Ghanaians deserves greater attention. About 75% of protein

in Ghanaian diet comes from starchy foods such as cereals, roots and plantains while intake of animal proteins which are rich sources of essential amino acids and micronutrients still remain deficient in the diet of vast majority of the populace (FAO-STAT, 2010). Therefore, at the national level more effort is needed to increase the proportion of animal protein by increasing accessibility and affordability to animal protein. Although the national daily average protein intake seems adequate for the whole population, nearly a quarter of school children are reported to be stunted (FAO, 2009). The prevalence rate of stunting was greater in rural areas (28%) against a prevalence rate of 13% in urban centers (GSS, 2007). Low levels of quality proteins in the diets in these children results in reduced cognitive development and increased susceptibility to diseases. Carbohydrate-rich diet with low quality protein increases mortalities and reduces growth during childhood. It also increases morbidity in adults by increasing susceptibility to infections, obesity and other non-communicable diseases (FAO, 2009). Therefore, improving quality protein intake seems still relevant in all age groups.

There are disparities in protein intake and distribution of malnutrition across the different regions of the country. According to FAO (2009), malnutrition characterized by stunting and wasting largely due to low level dietary protein intake is rated as “medium” at the national level, but higher in the former Northern Region (now North East, Savannah and Northern Regions) and Upper East Regions. Therefore strategic interventions are needed to improve protein intake particularly in Northern Ghana (FAO, 2009).

Interventions to address these disparities should focus on increasing accessibility and affordability of healthy animal protein to the larger population with particular attention to Northern Ghana (NG) and children.

1.2. Justification

Ghanaians have derived dietary animal proteins from diverse sources over centuries. Presently fish ranks the most consumed animal protein, while poultry ranks the most preferred meat (FAO, 2009). However both of these products largely come from imported frozen fish and meat which are listed among the top ten imported commodities to Ghana (UN , 2017). In the year 2013 Ghana imported 170,600 metric tons of frozen poultry products (UN Comtrade, 2016) worth 200.4 Million US Dollars (UN, 2017). The prices of these are subject to frequent increases due to higher rate of inflation (Al-Hassan *et al.*, 2014) making animal protein less affordable to populations particularly in the rural areas where poverty is more prevalent.

In this regard, the most appropriate intervention remains increasing and diversifying of domestic livestock and poultry production which currently account for only 8.9 % of Agricultural Gross Domestic Product (AGDP) and 1.1 % of total Gross Domestic Product (GSS, 2018). Greater preference of Ghanaian consumers for poultry informs all stakeholders to prioritize policy and technological interventions to revolutionize the poultry sub-sector in Ghana. Poultry include chicken, guinea fowl, duck and turkey with their significance varying based on the region.

The helmeted guinea fowl (*Numida meleagris*) is considered the most common poultry species in Northern Ghana (Agbolosu *et al*., 2012a), which is home to over 80% of domestic guinea fowl population (FAO, 2014). Helmeted guinea fowl is the most common type of fowl, within a group of Galliforms collectively known as “guinea fowls” (Frank

and Wright, 2006). Therefore the description "guinea fowls" refers to "helmeted guinea fowls (HLGF)" (*Numida meleagris*) throughout this thesis.

Guinea fowl production plays vital roles in the lives of guinea fowl farmers particularly in NG where protein related malnourishment is most prevalent. Almost every house hold in Northern Ghana keeps some guinea fowls (Dei and Karbo, 2004; Naazie *et al.*, 2007) while some raise them in larger numbers as profitable agribusinesses. The meat and eggs from guinea fowls are important sources of animal protein and other essential nutrients in rural households in NG (Agbolosu *et al.*, 2012b). However, in most cases, the primary purpose of rearing guinea fowls remains cash returns (Issaka and Yeboah, 2016). Rural farmers in the North recorded greater profits from guinea fowl rearing as compared to chicken (Avornyo *et al.*, 2016). Issaka and Yeboah (2016) indicated that guinea fowl farmers in Tolon and Busila North districts in Upper East recorded average net returns from 532 to 1750 USD per annum with a cost:benefit ratio of 8.2. Revenue generated from sale of meat and eggs is used to meet important financial needs such as paying school fees, medical treatment or as direct investment for crop farming (Avornyo *et al.*, 2016). Therefore, HLGF is not only an important protein source but plays a central role in the livelihood, wellbeing and socioeconomic growth of guinea fowl farmers and the general population in Northern Ghana.

Due to increasing preference for leaner meat, the demand is growing in the urban centers creating greater potential for commercialization than before (Issaka and Yeboah, 2016) with demand already exceeding supply (Karbo and Avornyo, 2006). Guinea fowl also has important socio-cultural importance as they are used in welcoming guests and payment of dowries (Teye and Gyawu, 2001; Issaka and Yeboah, 2016). Due to the aforementioned

nutritional, socioeconomic and cultural roles of local guinea fowls, the local guinea fowl varieties represent an important Animal Genetic Resource (AnGR) for the people of Northern Ghana.

The significance of local guinea fowl as an important AnGR goes beyond Northern Ghana for several emerging global trends. Due to the origin of HLGF in the Guinea Coast of Africa (Crowe, 1978), the guinea fowl populations found in NG are expected to represent most of their ancestral gene pool making them an important AnGR to the global guinea fowl industry which is growing (Nahashon *et al.*, 2006). Having survived extreme weather conditions prevalent in the Guinea Savannah zone, their genomes are also expected to be rich in adaptive genes that will make them an important genetic resource in the face of emerging threats of climate change that threatens other livestock species. Guinea fowls also exhibit greater resistance to common poultry diseases beyond the keet stage (Sayila, 2009) and can be reared with minimal farm inputs (Avornyo *et al*., 2016). This also is becoming an advantage amidst growing demand for organic poultry meat produced with minimal chemical inputs.

Amidst the socioeconomic significance, emerging advantages as an AnGR and increased demand due to recent preference for leaner meat, there are several challenges for commercialization of guinea fowl production in Africa (Moreki and Radikara, 2013). Slow growth rate and limited baseline data to facilitate genetic improvement programmes have been identified as two of the key challenges for the guinea fowl industry in tropical Africa (Agbolosu *et al.,* 2012b) although poultry farmers in African countries identified body size and faster growth among the most preferred traits to be improved in their breeds (Okeno *et al*., 2011).

The majority of guinea fowl farmers in NG (98%) depend on local varieties of guinea fowls (Avornyo *et al*., 2016) that have neither been characterized nor maintained as separate breeds. There is no evidence of dedicated breeding programmes to improve local guinea fowl breeds.

Selecting for faster growth has by far been the focus in majority of the poultry breeding programmes of the last century (ALBC, 2007). Poultry farmers desire breeds that grow faster to maximize their production capacity within a given period of time. Most long term breeding programmes that aimed at developing divergent lines for growth have used body weight at the eighth week as the selection criterion (Flisar *et al.,* 2014). Like many other quantitative traits, growth in poultry is influenced by genetic factors, environmental factors and their interactions (Lawrence and Fowler, 2012). It is a complex biological function tightly regulated by multiple neuro-endocrine factors. At the heart of this regulation and the coordination of environmental and genetic factors is the somatotropic axis (Kim, 2010).

Somatotropic axis modulates its physiological functions by two key proteins that share varying levels of similarities with insulin in structure and functions namely, the Insulin-like Growth Factor 1 (IGF1) and Insulin-like Growth Factor 2 (IGF2) (McMurtry *et al*., 1998). IGF1 and IGF2 bind to cell surface receptors on target cells and initiate signal transduction mechanisms that ultimately result in cellular reactions that culminate in growth at cellular, tissue and organ levels. Due to direct involvement of IGF1 and IGF2 in signal transduction of growth regulation, the genes that code for them are considered as important candidate genes for growth and body weight traits in poultry (Amills *et al*., 2003; Nie *et al.,* 2005; Xu *et al*., 2013). The polymorphic genotypes arising from these gene sequences have been linked to phenotypic variations of growth (Amills *et al*., 2003; Wang

et al., 2005) and body weight (Bian *et al*., 2008; Bhattacharya *et al*., 2015) making them ideal molecular markers for marker assisted selection schemes desiring genetic progress in growth.

Breed improvement can be achieved by breed replacement, cross-breeding with improved exotic strains or by improvement of local breeds through long term selection. Due to the relatively shorter time taken to achieve genetic progress and other factors, most African countries have largely opted for cross-breeding in past poultry breeding programmes (FAO, 2007a). Unfortunately, most of these programmes have failed due to low level of maintenance of genetic gain beyond a few generations, inbreeding and inability of offspring to adapt to conditions of tropical Africa and prevalent production systems (FAO, 2015). Therefore, the most sustainable option for breed improvement in African countries remain breed improvement through selection from indigenous breeds. Although African countries are endowed with rich AnGR, improving farm animals by selection programmes using indigenous AnGR is least considered largely due to the long time taken to achieve desired genetic progress.

Recent advances in the application of molecular genetic markers to complement phenotypic selection using Marker Assisted Selection (MAS) have the potential to significantly reduce the time taken and to increase selection intensity to achieve desired genetic progress (Eggen, 2012). However, application of MAS using markers such as Restriction Fragment length Polymorphisms (RFLPs) and Microsatellites remained low until the wide utilization of Single Nucleotide Polymorphisms (SNPs) (Stock and Reents, 2013). SNPs are polymorphisms arising from changes in DNA at single nucleotide positions (Vignal *et al*., 2002). Abundant genome-wide distribution, low cost and speed in

genotyping with high density genotyping platforms make SNPs ideal markers for Genomic Selection (GS) (Dekkers, 2012). Particularly with the reducing cost of genotyping, SNPs have the potential to be utilized in MAS in the developing world (FAO, 2007b). Although SNPs within functional regions such as exons, 5' and 3' untranslated regions (UTRs) are relatively rare compared to introns, their utility in MAS is high due to greater probability of association with phenotypes (Schmid *et al*., 2005).

Several workers have reported SNPs in IGF1 gene (Amills *et al*., 2003; Wang *et al.*, 2004; Nie *et al*., 2005; Gouda and Essawy, 2010; Pandey *et al*., 2013; Bhattacharya *et al*., 2015; Ilori *et al*., 2016) and IGF2 (Amills *et al*., 2003; Nie *et al*., 2005; Wang *et al*., 2005; Tang *et al*., 2010; Yan *et al*., 2017) in chicken, the most studied avian model. Associations between these SNPs and growth rate have also been reported (Amills *et al.*, 2003; Zhou *et al.*, 2005; Pandey *et al.*, 2013; Bhattacharya *et al*., 2015). However, there have been no previous reports on SNPs within IGF1 gene and IGF2 gene in guinea fowls. Recent release of Whole Genome Sequence of guinea fowl by Vignal *et al.* (2017) is a major milestone in realizing the potential of genomic selection in this species. Therefore, identification of SNPs associated with growth in local guinea fowls will make significant contributions to facilitate MAS. Identification of these SNPs will also facilitate representation of genotypes present in local birds in high density SNP panels in the future to make genomic selection useful for breed improvement programmes in Africa.

Therefore, this study seeks to identify SNPs within exonic regions of IGF1 and selected regions in IGF2 genes in guinea fowls and to determine associations between them and early growth and body weight traits in three indigenous guinea fowl populations originating from Northern Ghana (NG).

1.3. Objectives

1.3.1. Main Objective

The main objective of the study was to identify Single Nucleotide Polymorphic DNA markers within exonic regions of Insulin-like growth factor 1 gene (*IGF1*) and selected regions of Insulin-like growth factor 2 gene (*IGF2*) in local guinea fowls (*Numida meleagris)* from the three regions of Northern Ghana (i.e Upper-East, Upper-West and former Northern Region) and to determine their associations with early growth and body weight traits during the keet and grower stages.

1.3.2. Specific objectives

The study was conducted with the following specific objectives

1. To compare three main guinea fowl populations from Northern Ghana (Upper East Region, former Northern Region and Upper West Region) for early growth and body weight traits during the keet and grower stages.
2. To identify SNPs within exonic regions of guinea fowl IGF1 gene (*gIGF1*) among the three populations of local guinea fowls of Northern Ghana and to determine their distribution among the three populations.
3. To identify SNPs within guinea fowl IGF2 gene (*gIGF2*) among three local guinea fowl populations from Northern Ghana and to determine their distribution among the three populations.
4. To establish associations between the body weight and early growth traits with the identified SNPs within *gIGF1* and *gIGF2* in local guinea fowls from the three populations of Northern Ghana.

CHAPTER 2

LITERATURE REVIEW

2.1 Helmeted guinea fowl raised in Northern Ghana

The helmeted guinea fowl (*Numida meleagris*) is an important bird indigenous to West Africa. Although there have been reports of guinea fowls domesticated in ancient Egypt (Newbold, 1926), the origin of present day domesticated guinea fowl can be traced to the Coast of Guinea that spans the present day West Africa (Moreki and Radikara, 2013). They were domesticated by the indigenous African tribes during their transition from a pastoral to an agricultural lifestyle. They were introduced to Europe during navigational exploits of Prince Henry of Portugal during the 15th century and were then taken to Great Britain, from where they spread all over the world (Newbold, 1926). Guinea fowls still remain an important Animal Genetic Resource (AnGR) to populations in West Africa and Africa at large. In Ghana, the majority of them are raised in Northern Ghana (NG) where guinea fowl production plays vital nutritional, socio-economic and cultural roles in the livelihoods of local people (Avornyo *et al*., 2016).

Taxonomically they belong to the Class Aves, Subclass Neognathae, Infraclass Galloanserae, Order Galliforms, Family Numididae, Genus *Numida* and species *meleagris* (Plate 1). The helmeted guinea fowls are considered the most common poultry bird in Northern Ghana (Agbolosu *et al*., 2012a) and remain the focus of this thesis.

Plate 1. The helmeted guinea fowl (*Numida meleagris*)

Source: New Zealand Rare Breeds (Website: www.rarebreeds.co.nz)

2.1.1. Distribution of local guinea fowls in Ghana

The guinea fowl is one of the most important indigenous poultry species in Ghana and Africa at large. In 2009, an estimated 2,574,996 guinea fowls were raised throughout Ghana (FAO, 2014). However, over 80% of the guinea fowls produced per annum in Ghana are concentrated in the northern part of Ghana where this bird is an integral part of the farming system (Dei and Karbo, 2004). The populations of local guinea fowls in the Upper West Region, Upper East Region and former Northern Region were estimated to be 59,360, 622,616 and 1,414,649 respectively in the year 2009. A considerable level of guinea fowl production also takes place in the former Volta and former Brong Ahafo regions (Ahiagbe *et al*., 2014).

2.1.2. Key guinea fowl production practices in Ghana

An understanding of production practices of livestock farmers in a breeding programme is crucial to ensure sustainability (FAO, 2007a). As production practices essentially define the environment in poultry production, knowledge on production practices is also crucial to interpret observations during animal experiments.

Due to the dynamic nature of farmer practices, a recent study conducted by Avornyo *et al.* (2016) provides insight into current status of guinea fowl production practices in NG. According to Avornyo *et al.* (2016), over 95% of farmers provided some form of housing including mud huts, wooden coops or cemented structures. Poor housing has been associated with low productivity in guinea fowls. All farmers in the Upper East Region provided supplementary feeding while a majority of farmers provided supplementary feeding in Northern (former) and Upper West Regions. Termites and Maize were the most common supplementary feed ingredients provided in both former Northern and Upper

West Regions, respectively, during keet stage and adult stage. In the Upper East Region, keets were fed with millet and termites while the growers and adults were fed with maize and millet. About 80% of farmers provided water to the flock. The majority of farmers in NG swept the coop once a day but very few changed litter and provided feed in feeders. The number of farmers that adopted best practices of biosafety was minimal (Avornyo *et al*., 2016).

Farmers in NG depended on ethno-veterinary medicines for managing their flock health. A larger proportion of farmers (35%) in Upper East dewormed their birds with orthodox medications compared to Upper West (27%) and former Northern Region (19%). However, the proportion of farmers that followed a vaccination schedule was higher in the Upper West Region (27%) compared to 7% and 14% farmers in the Upper East and former Northern Region, respectively (Avornyo *et al*., 2016).

2.1.3. Common breeding practices and breeds adopted by guinea fowl farmers in Northern Ghana

The vast majority (98%) of guinea fowls raised in NG are local guinea fowls that have probably been with the indigenes over centuries. A few breeding programmes to disseminate exotic breeder stock conducted in these regions accounted for the remaining 2% of the population which included 1% each of exotic breeds and cross-bred guinea fowls (Avornyo *et al*., 2016). No reports on specialized breeds developed and maintained by any of the key stakeholders of guinea fowl AnGR management in Ghana were found. Unavailability of good quality keets for replacement of stock has been considered a challenge for intensification of guinea fowl farming in the country (Teye and Adam, 2000; Avornyo *et al.*, 2016).

In the case of production systems prevailing in NG, guinea fowls are largely bred by the parent stock maintained by the farmers themselves or by incubating the eggs bought from fellow farmers or rarely from the market and incubated using a domestic fowl or using artificial incubators. In limited areas farmers practiced a male:female sex ratio of 1:1 while majority adopted a sex ratio of 1:5 or 1:3. Sex was determined by phenotypic appraisal based on the helmet, body size, appearance of legs and feathers (Avornyo *et al*., 2016). Farmers did not maintain a separate breeder stock under best practices of breeder stock management in any of the areas (Avornyo *et al*, 2014; Avornyo *et al*., 2016).

2.1.4. Performance of local guinea fowls under production systems common in Northern Ghana

Performance appraisal of a given ecotype or a breed is important during phenotypic characterization. It also forms most of the field work in a breeding programme and experiments with livestock species. Exact traits of interest to be studied depend on the objectives of the research or the breeding programme and stage of the production cycle. Important performance traits to be appraised during the early stage of poultry production include body weight, growth rate and survivability (ALBC, 2007; FAO, 2007a).They are discussed below with reference to guinea fowls raised in NG.

2.1.4.1. Body weight

Body weights measured at specific time intervals have been measured as key performance traits in a large number of appraisal experiments during characterization of breeds. Body weight is also one of the growth dependent traits that can be easily and accurately measured (ALBC, 2007; Ayorinde, 2007). Most of the long term breeding programmes have utilized

body weight at the eighth week to select divergent lines for growth in chicken (Flisar *et al.*, 2014). Like any other quantitative trait, body weight measured at specific ages is influenced by the genotype and the environmental factors largely due to production conditions at the farm (Oke *et al*., 2004; Ayorinde, 2007).

Agbolosu *et al*. (2012a) observed significant differences in overall body weight of guinea fowls from Upper East, Upper West and former Northern Region of Ghana raised at a Research station, under intensive production from eight to 18 weeks. The birds from Upper East Region recorded the highest mean body weight. However, they did not report on body weights at time intervals during early growth of guinea fowls. Body weight at eight weeks has been measured during several comparative performance experiments with young guinea fowls. Dei *et al*. (2009) observed a mean live weight of 379.2 g for guinea keets brooded in confinement against 102.2 g for those raised with the foster mother under extensive system at eight weeks in some sample locations in former Northern Region. In another experiment, Mohammed and Dei (2017) reported 119.2 g and 381.6 g body weight at eight weeks for local guinea fowls in former Northern Region brooded under extensive system and intensive system, respectively. However, the body weights measured during the two different experiments cannot be compared due to variation in experimental conditions. Outside Africa, Khairunnesa *et al*. (2016) recorded 377.7 g at eight weeks in local guinea fowls raised under intensive system in Bangladesh, while Nahashon *et al.* (2006) observed a mean body weight of 787.05 g at eight weeks for a meat variety of guinea fowls selectively bred for faster growth in USA.

2.1.4.2. Growth traits

Although growth is a complicated trait to be quantified, daily weight gain provides an accurate and convenient expression of growth on a quantitative scale and has been used by many authors to measure growth. Agbolosu *et al*. (2012a) compared daily weight gain in guinea fowls from Upper East, former Northern and Upper West regions between eight to 18 weeks of production under intensive system and found no significant differences in growth between eight to 18 weeks. Mohammed and Dei (2017) recorded daily weight gains of 2.43 and 7.79g/bird/day, respectively, for the first eight weeks of local guinea fowl keets brooded under extensive and intensive systems, respectively. Relatively higher rates of weekly weight gain in keets managed under intensive system were also reported by others (Naandam and Issah, 2012). Khairunnesa *et al.* (2016) reported weekly weight gains of 15.7, 12.73, 10.57 and 23.2 g/week/bird for the first, second, third and fourth weeks after hatch for guinea fowl breeds raised under intensive management in Bangladesh. Nahashon *et al*. (2006) recorded remarkably high growth rates of 34.72, 61.34, 90.93 and 110.71 g/week from the first to fourth week respectively, in selectively bred pearl guinea fowls in USA. Protein content of feed is one of the factors affecting average daily weight gain in guinea fowl keets (Avornyo *et al*., 2013).

2.1.4.3. Feed intake

Growth and feed intake are quantitative traits that largely influence each other. Birds that grow bigger eat better and vice versa (Oke *et al*., 2004). Agbolosu *et al*. (2012a) observed significant differences between feed intake in guinea fowls from Upper East, former Northern, and Upper West related to their size. Avornyo *et al.* (2013) recorded an average

feed intake of 7.04g/keet/day during keet stage of production on a diet with 24% crude protein. Performance at this protein inclusion rate was better compared to other treatments with local guinea fowls from Northern Ghana.

2.1.4.4. Survivability

Survivability is one of the key factors not to be overlooked while aiming at genetic gains in other productive traits. It is also an important economic trait as mortalities reduce the profit margins in poultry production (ALBC, 2007). Survivability as a trait is even more important in local guinea fowl keets raised under the semi-intensive production system as losses due to keet mortalities has been described as the major challenge to guinea fowl production in Northern Ghana from past (Teye and Adam, 2000) to present (Avornyo *et al*., 2016). High mortality levels during the first eight weeks, with great reduction thereafter has also been reported among local guinea keets in other African countries (Nwagu and Alawa, 1995; Dahouda *et al.*, 2007) and is attributed to bad weather, poor feeding, poor housing and diseases (Dahouda *et al.*, 2007). However, confining the birds with provision of feed, water, heat and light under intensive management significantly reduced keet mortality (Dei *et al*., 2009; Naandam and Issah, 2012; Mohammed and Dei, 2017). Even with better brooding, high keet mortality still remains a challenge in Northern Ghana (Avornyo *et al*., 2016). Agbolosu *et al.* (2012a) recorded limited mortalities beyond eight weeks in guinea fowls from Upper West, former Northern and Upper East Regions in the increasing order of survivability rate by regions.

2.2 Molecular genetic markers and their application in animal breeding

Molecular genetic markers are DNA sequences present in the genomes of living organisms that act as molecular indicators of another DNA sequence, an attribute such as a phenotype or phylogenetic relationship (FAO, 2007a). Since the first description of Restriction Fragment Length Polymorphic markers (RFLPs) in tomato, a host of other molecular genetic markers have evolved including microsatellites, Amplified Fragment Length Polymorphic DNA markers (AFLPs), Randomly Amplified Polymorphic DNA markers (RAPDs), Single Nucleotide Polymorphisms (SNPs) and Expressed Sequence Tags (ESTs) (FAO, 2007b). They differ in relative specificity of location, relative abundance in the genome, transferability across species, reproducibility, degree of dominance and the method of genotyping (Duran *et al*., 2009).

2.2.1. Restriction Fragment Length Polymorphisms (RFLPs)

Restriction enzymes are enzymes of bacterial origin that cleave DNA at specific sequences called restriction sites to produce blunt ended or sticky ended DNA. Based on the distribution of restriction sites on genomic or amplified DNA, restriction digestion results in fragments of different sizes that can be resolved by gel electrophoresis to generate a unique finger print that can be blotted to nylon membranes. A target marker can be detected by fluorescent or radio labelled probe (Sambrook and Russell, 2001). RFLPs work well for loci with limited polymorphism such as Mendelian traits. High degree of inbreeding in livestock makes RFLP less informative in Marker Assisted Selection (MAS) (FAO, 2007b). SNPs arising from substitutions and indels can result in gain of a restriction site or loss of a restriction site giving rise to different genotypes evident from different fingerprints. Nagaraja *et al*. (2000) reported a A>C transversion within the promoter region

of *IGF1* in White Leghorn chicken which was also subsequently reported by Wang *et al*. (2004) in Wanzhai Yellow breeds and by Pandey *et al*. (2013) in coloured Indian broiler chicken. In guinea fowls, however, there has not been any reported case of polymorphisms in *gIGF1* detected by PCR-RFLP.

2.2.2. Randomly Amplified Polymorphic DNA (RAPDs)

Instead of using target specific forward and backward primers as in specific PCR, RAPDs are amplified products generated by PCR using single primers with arbitrary sequences called random primers. Fragments resulting from amplification from primer complementary sites located close enough yield RAPD fingerprints on agarose gels. A unique and consistent RAPD fingerprint linked to a given QTL or a gene can be used as a marker for that locus (Welsh and McClelland, 1990). Although random primers are relatively inexpensive, primer annealing differs with slight modifications in PCR conditions and quality of genomic DNA making it less reproducible. RAPDs have been used to study genetic diversity in various poultry species including guinea fowl populations in Poland (Bawej *et al*., 2012).

2.2.3. Simple Sequence Repeats (SSRs)

Variable Number of Tandem Repeats (VNTRs) or Simple Sequence Repeats (SSRs) are DNA sequences made up of tandemly repeated di-, tri-, tetra-, penta- or hexa- nucleotides. SSRs are mostly found in noncoding sequences. They are classified as microsatellites and minisatellites based on the length of repeat unit (Gholizadeh and Mianji, 2007). The high rate of polymorphism, relative abundance, codominant inheritance and convenient genotyping made possible by automated sizing and genotyping make them versatile

markers for large number of diversity studies in many poultry species (Duran *et al*., 2009). However, due to their limited association with phenotypes and limited understanding on their influence in rate of gene expression, their application in MAS in the past has been limited (Dekkers, 2012). Microsatellite loci associated with resistance to Marek's disease (McElroy *et al.*, 2005) and antibody response (Yonash *et al*., 2001) have been described in chicken. A set of novel microsatellites was developed by Botchway *et al*. (2013) using 454 Next generation sequencing (NGS) for potential use in guinea fowls and was used to study genetic diversity of local guinea fowls in Ghana. Weimann *et al.* (2016) used microsatellite markers to compare domestic and wild populations of guinea fowls in Sudan.

2.2.4. Single Nucleotide Polymorphisms (SNPs)

SNPs are variations arising from a change in nitrogenous base at a specific single base pair location in the genome (Schmid *et al.*, 2005). Based on the type of variation they can be described as transitions, transversions or indels. Transitions arise from a substitution of a purine with another purine as in Adenine (A) with Guanine (G) or a pyramidine with another pyramidine as in substitution of Cytosine (C) with Thymine (T). A transversion replaces a purine with a pyramidine or vice versa resulting in a greater change in DNA structure and is likely to bring about a more drastic change than in the case of transitions (Emara and Kim, 2003). Considering that mutations are random events, theoretical frequency of transversions should be twice that of transitions. However, the observed ratio of transitions to transversions is 1.4:1 in mammals (Collins and Jukes, 1994) and 4:2.3 in chicken (Vignal *et al*., 2002). This is probably due to the ease of simple chemical modifications such as deamination of 5-methyl cytosine to thymine which also makes C to

T transitions the most common mutations (Schmid *et al*., 2005). Indels arise from either insertion or deletion of one or few base pairs at a specific single base location of a genome.

SNPs are the most abundant type of markers in the genome found in intergenic bulk DNA, introns, coding regions and noncoding regions of exons such as the 5' untranslated region (5'UTR) and 3' untranslated region (3'UTR) (Duran *et al*., 2009). SNPs occurring within coding regions may be critical as they can bring about an alteration in the amino acid giving rise to a non-synonymous mutation as opposed to a synonymous mutation caused by a SNP. A SNP that does not bring about a change in the amino acid in the associated codon is called a synonymous mutation (Emara and Kim, 2003). Due to their involvement in gene function, SNPs present in coding and non-coding regions of exons, are likely to be associated with phenotypic variations than SNPs found in nonfunctional segments of the genes (Schmid *et al*., 2005). High probability of transitions, third group degeneracy of the genetic code and other factors make genomes inherently resistant to mutations thereby increasing the stability of genetic material. Nevertheless, they occur, giving rise to genetic and phenotypic diversity which probably harbor opportunities for survival in changing environments.

Due to their genome-wide distribution, greater abundance, relative stability during inheritance, greater association with phenotypic polymorphisms and convenient genotyping in a massively parallel manner using high density SNP genotyping platforms at a relatively lower cost, SNPs make ideal markers for GS (Stocks and Reents, 2013). Discovery of SNPs involves comparison of sequences generated from Sanger sequencing or NGS of target DNA from genomic DNA clones, PCR amplified gene targets or Expression Sequence Tags (ESTs). Animals can be genotyped for SNPs from direct

sequencing, PCR-RFLP, Single Strand Confirmation Polymorphism analysis or Nucleotide Ligation Assays and many emerging methods (Duran *et al*., 2009). NGS which allows simultaneous sequencing of multiple fragments in a massively parallel manner has enabled completion of Whole genome sequencing of many poultry species including chicken (International Chicken Genome Consortium, 2004), turkey (Dalloul *et al*., 2010), and guinea fowls (Vignal *et al*., 2017) and has accelerated rate of SNP discovery genome-wide.

2.2.5. Applications of molecular genetic markers in animal breeding

Molecular genetic markers have diverse applications in genetic improvement of farm animals including parentage determination, genetic distance estimation, determination of twin zygosity and freemartinism, early sexing of monomorphic birds, identification of disease carriers and, most importantly, Marker Assisted Selection (FAO, 2007a). Genomic selection include the most recent application of Marker Assisted Selection.

2.2.5.1. Marker Assisted Selection (MAS)

Most of the genetic improvement in livestock species over the last few centuries was possible through phenotypic selection based on breeding values estimated with phenotypic measurements (Estimated breeding values; EBV; Dekkers, 2012). Genetic progress made during selection is proportional to accuracy and selection intensity but inversely proportional to the generation interval. Most of economic traits are regulated by a large number of loci scattered throughout the livestock genomes and environmental interactions (Falconer and Mackay 1996). Such loci, that act together to bring about expression of phenotypic traits are cumulatively referred to as Quantitative Trait Loci (QTL). Molecular markers that are proved to have statistical association with a given QTL are informative of

the presence of that QTL in a selection candidate. Complementing phenotypic selection with information available from markers is referred to as Marker Assisted Selection (Dekkers, 2012). Application of MAS using genome-wide high density markers provide the basis for genomic selection.

2.2.5.2. Genomic selection (GS)

The advent of NGS which enables sequencing of whole genomes in massively parallel manner, has made Whole Genome Sequencing (WGS) rapid and cost effective (Mardis, 2008). WGS laid the foundation for a new era of molecular genetics with the introduction of genome-wide SNP markers. Soon, high density genotyping platforms became available for simultaneous genotyping of an individual animal for thousands of SNPs across the genome. The first such platform was the Illumina Bovine SNP 50 Bead Chip (Illumina Inc.), optimized for both dairy and beef cattle (Dekkers, 2012). Presently, such panels are available for chicken, sheep, goat and horse. Genotyping data routinely generated with SNP panels have been utilized to calculate Genome Enhanced Breeding values (GEBV). Application of genome-wide SNP-based markers to complement phenotypic selection has come to be known as Genomic selection (GS) or genome-wide selection. Due to simultaneous use of thousands of SNPs, application of GS has increased selection accuracy in breeding programmes. Improvements of up to 60% in accuracy of predictions have been reported with the use of high density panels in dairy cattle breeding (Stock and Reents, 2013). Genomic selection has also reduced the demand for very large reference populations with extensive pedigree data. Breeders have also been able to significantly reduce generation interval to increase genetic gain (Goddard and Hayes, 2009). Ability to predict breeding values for traits that were previously difficult to measure including those with

low heritabilities, traits measured only after slaughter and traits that require challenging birds with infectious diseases are among other benefits of GS (Eggen, 2012). Presently, breeding organizations dominating dairy cattle breeding routinely carry out SNP genotyping of Holstein cattle to aid selection of "genomic" bulls and females for selective breeding primarily through Artificial Insemination. However uptake of genomic selection has been limited in developing countries for several reasons. Currently available SNP chips have been developed mostly with temperate breeds with limited representation of those from the developing world. This limits their ability to genotype less characterized indigenous breeds and composite breeds which are rather widespread in the production systems prevailing in these countries. Although GS limits the need for large reference populations with extensive pedigree data its implementation still depends on accurate phenotypic recording schemes. Most developing countries lack infrastructure for phenotypic recording. Amidst progress made in reduction of cost in genotyping, high density SNP chips still remain expensive for developing countries while most countries still await laboratory infrastructure for genotyping and computational infrastructure for data analysis (Montaldo *et al*., 2012).

2.2.5.3. Marker Assisted Introgression (MAI)

Introgression refers to a breeding strategy that involves transferring a set of target favourable alleles from a donor population to a recipient population by a series of crosses and removing unfavourable alleles by a series of back crosses. Segregation of target genes to be introgressed or accompanying genes can be tracked using markers associated with the respective alleles, where introgression is marker assisted, to increase selection intensity, accuracy and to reduce generation interval (Visscher *et al*., 1996).

2.2.5.4. Mapping QTLs

Before the advent of WGS and discovery of large number of SNPs, QTLs responsible for traits of economic importance in farm animals were mapped with linkage analysis and Linkage Disequilibrium (LD) with markers such as microsatellites. Establishing statistical relationships for large numbers of SNPs in the genome with traits within a sample using Genome-wide Association studies (GWAS) has only been able to identify a limited number of genes that follow Mendelian inheritance so far (Goddard and Hayes, 2009). Although the advent of NGS has accelerated SNP discovery, paving way to GWAS and GS, QTL mapping using GWAS has several limitations including Beavis effect arising from false positive associations of SNPs that are in LD with candidate SNPs (Xu, 2003).

2.2.5.4. Assessment of genetic diversity

While the realization of the full potential of MAS is awaiting mapping of QTLs, assessment of genetic diversity remains the widest application of molecular markers so far (FAO, 2007a). Measurement of genetic diversity is important to select individuals that are genetically distant to establish reference populations prior to breeding schemes and to inform conservation decisions (FAO, 2007b). Microsatellites have been the most widely used markers in genetic diversity studies (Gholizadeh and Mianji, 2007), including guinea fowls in Ghana (Botchway *et al*., 2013) and Sudan (Weimann *et al*., 2016). SNPs, when available genome-wide, are also very useful to ascertain genetic diversity within and between populations. Vignal *et al*. (2017) assessed the diversity of a domesticated French guinea fowl breed and indigenous guinea fowls from West Africa using genome-wide SNPs after whole genome sequencing. Their findings provided further evidence for the

domestication scenarios of helmeted guinea fowls. In diversity studies, markers are used to genotype individuals to ascertain three major hierarchical measures of heterozygosity that can be further used to calculate three main indices based on F statistics including inbreeding coefficient (F_{IS}), fixation index (F_{ST}) and overall fixation index (F_{IT}). According to Hartl and Clark (1997) F_{ST} value ranges of 0 to 0.05, 0.05 to 0.15, and 0.15 to 0.25 indicate little, moderate and great genetic variation, respectively.

2.2.6. Candidate gene approach

GWAS establishes the statistical association between large numbers of SNPs present genome-wide with the traits of interest without assigning the SNPs within the genome. Thus all SNPs that are in LD at multiple loci appear to be associated with the traits creating false positive associations and wrongfully meriting a selection candidate due to absence of linked SNPs that have no actual biological influence on that trait (Goddard and Hayes, 2009). Therefore it is important to decipher the SNPs at genic level and possibly determine the molecular basis for the observed association using the candidate gene approach. Candidate gene approach involves identification of genes based on their physiological role, studying the genetic polymorphisms and their associations with the trait of interest on a gene by gene basis (Zhu and Zhao, 2007). This approach enables identification of less number of more informative SNPs to make more accurate predictions with larger number of breeds.

2.3. Growth and regulation of growth in poultry

Growth is a complex process that occurs in all living organisms at cellular, tissue, organ and systemic levels. For the want of a quantitative expression, it can be described as the

increase in size per unit time (ALBC, 2007). Growth is a continuous process that occurs from conception to maturity controlled by integrated regulation of cell proliferation and differentiation. Although it is far from a mere quantitative increase in body size, increase in body weight per unit time expressed as daily weight gain or weekly weight gain remains the simplest and most widely used quantitative measure of growth (Lawrence and Fowler, 2012).

It is one of the most important quantitative traits in farm animal genetics and breeding affected by the genotypes of so many growth related genes and farm animal management including nutrition and health status (Ayorinde, 2007). Virtually all breeding programmes use growth, at least, as one of the key parameters while faster growth remains the main breeding objective for a large number of poultry breeding programmes aiming at improving meat traits (ALBC, 2007). In general, due to high degree of phenotypic diversity within local breeds, they record lower mean growth rates compared to specialized breeds (FAO, 2007a).

Poultry growth is divided into five phases including embryonic, post-hatch, juvenile, pubertal and adult stage (Kim, 2010). Guinea fowls follow a sigmoid pattern of growth like many other poultry species (Nahashon *et al.,* 2006). Published literature on growth curve parameters in local Ghanaian guinea fowls is not available in the public domain. In tropical Africa the first eight weeks of guinea fowls are considered the critical period in guinea fowl production cycle (Avornyo *et al*., 2016) due to high level of mortality recorded during this period.

2.3.1. Regulation of growth in Poultry

Growth in poultry is regulated by a complex network of proteins and their interactions with each other. One of such polypeptide hormones, the Growth Hormone (GH), exerts some growth stimulating activity by directly acting on target tissues or to a larger extent through somatotropic axis (Kim, 2010). This molecular regulatory framework is initiated at the hypothalamus of the brain in birds, which produce Growth Hormone Releasing Hormone (GHRH) which binds to receptors on somatotrophs, a specialized type of cells present in the anterior pituitary gland. This binding results in release of GH by anterior pituitary (Scanes, 1989). GH can bind to Growth Hormone Receptors (GHRs) at target tissues that can initiate molecular mechanisms that ultimately result in growth. One of the key target tissues is the liver which expresses GHRs from embryonic stage till puberty (Ahmed and Farquharson, 2010). GH binding to GHRs in the liver amplifies GH signal by releasing IGF1, another growth hormone that plays a pivotal role in the somatotropic axis. However IGF1 can be synthesized by some tissues in a GH independent manner during embryonic growth and beyond (Kühn *et al.*, 2002). The somatotropic and thyrotropic axes are tightly linked and have coordinated influence on growth in poultry (Kim, 2010). These key regulatory factors themselves are proteins and are expressed by transcription and translation of their genes. Expression levels of these factors vary with the age, growth stage and are based on signals from intermediary metabolism (McMurtry, 1998). Figure 1 provides a schematic representation of expression of key modulators of growth at different growth stages in poultry according to Kühn *et al*. (2002).

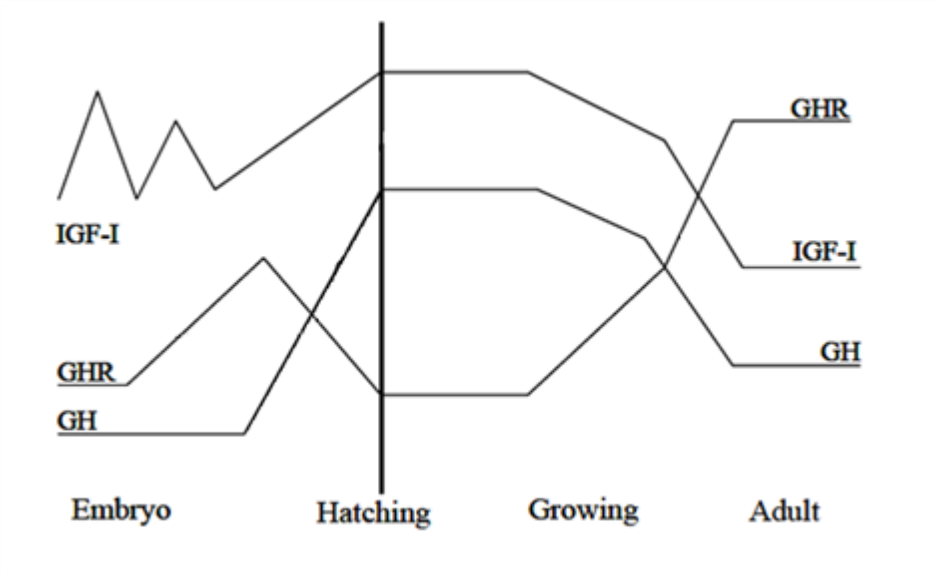


Figure 1. Pattern of expression of GH, GHR and IGF1 during various stages of growth in poultry (Figure not drawn to scale; modified from Kühn *et al.*, 2002)

2.3.2. Insulin-like growth factor system

Existence of a possible intermediary substance through which GH exerted its activity was first hypothesized by Salmon and Daughaday (1957) while setting up an *in vitro* assay for GH. They named this factor "Sulphation factor (SF)" as it increased the uptake of Sulphate into cartilage tissue in hypophysectomized (hypox) rats. Later, non-suppressible insulin-like activity (NSLA) of human plasma extracts, described by Froesch *et al.* (1966) was linked to the Sulphation factor. Marquart *et al.* (1981) purified and established the primary structure of a polypeptide from rat liver cells with "multiplication stimulating activity (MSLA)". As a result of improvements in radioimmunoassays, DNA sequencing and other concurrent advancements, the existence of a group of hormone-like polypeptides similar to insulin in structure and function became clear. These polypeptides commonly described as Insulin-like Growth Factors (IGFs) at present, consist of two main proteins namely IGF1 and IGF2 and are found across vertebrate taxa (Kim, 2010). Mammalian counterparts are the most studied and characterized IGFs among vertebrates compared to avian IGFs. The first and most studied avian IGFs are chicken counterparts.

Although IGF1 and IGF2 are the key growth factors within the IGF system, they do not work in isolation. IGF1 and IGF2 interact with other proteins including their receptors and IGF Binding Proteins (IGFBPs) to bring about IGF mediated growth regulation through signal transduction. IGFBPs are specialized proteins that bind to IGF1 and IGF2 with high affinity to form ternary complexes (McMurtry *et al*., 1997). There have been reports of, at least, seven IGFBPs of which IGFBP3 is dominant. IGFBPs have multiple roles in transporting IGFs in blood, localizing IGFs within extracellular matrix for paracrine and autocrine functions. They also modulate IGF binding to their receptors and influence

downstream signal transduction of IGFs (Clemmons, 1993). IGFBPs are subjected to complex post-translational modifications of phosphorylation, proteolysis and glycosylation most of which up or down-regulate their binding affinity to IGFs and their growth promoting activities (Lei *et al*., 2005). Studies on IGFBP expression show complex patterns of IGFBP2, IGFBP3, IGFBP5, and IGFBP7 that vary depending on the embryonic day, stage of post-hatch growth and diet in chicken (Lu *et al*., 2010). Signal transduction of IGF1 is initiated by specific binding to Insulin-like growth factor 1 receptor (IGF1R), which belongs to the family of tyrosine kinase receptors (McMurtry, 1998).

2.3.3. Candidate genes for growth

Literature reviewed in past sections reveals the complexity of regulation of growth which includes at least the GH, Thyrotropic and Somatotropic axes. Each of these pathways are implemented by a complex network of proteins including GH, GHR, IGF1, IGF2, IGF1R and IGFBPs. These proteins are transcribed by genes designated as *GH, GHR, IGF1, IGF2, IGF1R* and IGFBP gene family, respectively (Nie *et al*., 2005). Although their degree of significance vary based on their function, all these genes that transcribe proteins that constitute the molecular framework for growth regulation become candidate genes for growth. Therefore, they form important components of growth linked QTLs (Xu *et al*., 2013).

Due to their roles as candidate genes for growth, polymorphisms at the DNA level have been investigated for *GH* (Nie *et al*., 2005), *GHR* (Ouyang *et al*., 2008), *IGF1* (Nagaraja *et al*., 2000; Nie *et al*., 2005; Bhattacharya *et al*., 2015), *IGF2* (Wang *et al*., 2005; Yang *et al*., 2017) *IGF1R* (Lei *et al*., 2008) IGFBP gene family (Lei *et al*., 2005).

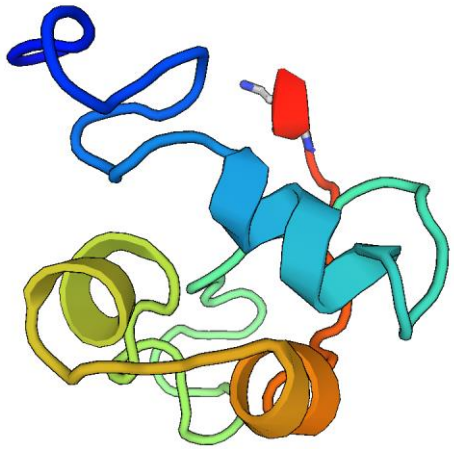
2.4. Insulin-like Growth Factor 1 (IGF1)

IGF1 is one of the most important modulators of growth and metabolism in metazoans. Biochemically, it is a single chain polypeptide with a unique tertiary structure evolved to perform its physiological functions through endocrine, autocrine and paracrine activities. At cellular level, IGF1 achieves this by playing a pivotal role in signal transduction leading to increased metabolism and cell proliferation.

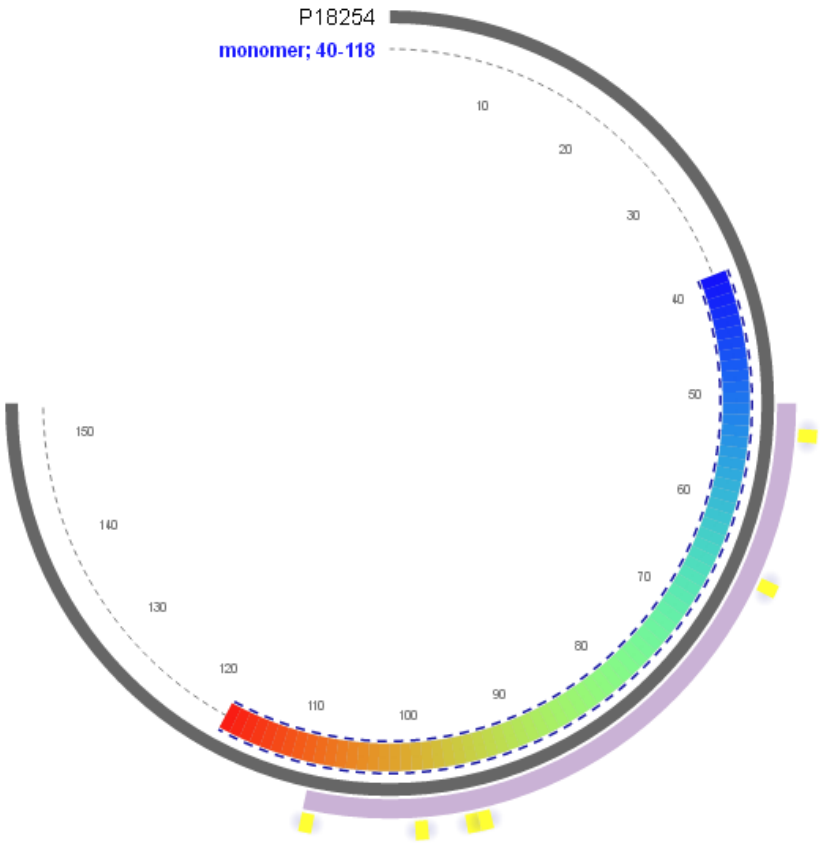
2.4.1. Structure of IGF1 proteins

Human IGF1 (hIGF1) was the first and most widely studied IGF1 across vertebrate taxa and provided insight into IGF1s of other species due to high degree of conservation of amino acids in the primary structure across species. Three dimensional structure of hIGF1 was first proposed by Blundell *et al.* (1977). Human IGF1 is a compact globular molecule. Within the tertiary structure there are two typical right handed helices and an additional less organized helix between the 54^{th} and 60^{th} amino acid residues. A connecting peptide spans between residues 30 and 41. Of great importance of the amino acids to maintain accurate protein folding and structural integrity are the four Cysteine residues which participate in disulfide bond formation. While the core of IGF1 is largely hydrophobic, the surface is rich in polar amino acids such as Arginine, Glutamate and Aspartate, part of which are from the connecting peptide which lies on the surface. These covalent and non-covalent intramolecular bonds between the amino acids and their interactions with the solution results in a three dimensional confirmation that fits their interaction with the receptors, binding proteins and provide the structural basis to its physiological functions (Blundell *et al*., 1977).

Chicken IGF1 (cIGF1) is perhaps the most studied IGF1 protein among avian species. It was first purified and partially sequenced by Dawe *et al.* (1988). It weighs about 7738 daltons compared to the human IGF1 that weighs about 7649 daltons (Ballard *et al.*, 1990). Mature cIGF1 is made up of a single polypeptide chain of 70 amino acids and shares significant homology with hIGF1 (McMurtry *et al.*, 1998) with only amino acid substitutions at eight residues (Ballard *et al.*, 1990). Unlike the hIGF1 of which secondary and tertiary structures are well understood, published literature on the secondary structures present in cIGF1 and its three dimensional domain structure is scanty. However, high degree of homology between cIGF1 and hIGF1 and *in silico* protein modelling can be used to predict the secondary structures and tertiary structure of cIGF1. The SWISS-MODEL generated from amino acid sequence for cIGF1 (Bienert *et al.*, 2017) is given in Figure 2 (a). PreproIGF1 is the primary polypeptide (Figure 2b) translated from ribosomes on endoplasmic reticulum of IGF1 secreting cells. There is no literature on post-translational modifications of avian IGF1. However organization of preproIGF1 suggests a general scheme for post-translational modifications. Post-translational modifications cleave the propeptides leaving the mature protein that undergoes accurate protein folding to acquire the three dimensional domain structure critical for accurate binding with its receptor and associated proteins (Nelson and Cox, 2005). Propeptides do not have a direct involvement in mature protein function but influence the rate of post-translational modifications and bioavailability of the final protein (Hede *et al*., 2012). Polymorphisms within DNA coding for specific regions of a protein can result in amino acid alterations that may influence the tertiary structure, its interactions with the receptor and binding proteins, ultimately resulting in variations in biological function.



(a)



(b)

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MODEL  LTLTSSAAAGPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLEMYCAPIKPPKSA 118
3lri.1.A MPLSSLFVNGPRTLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRACQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA  83
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(c)

Figure 2. Structure of chicken IGF1 protein (Source, https://swissmodel.expasy.org/workspace): (a), Three dimensional structure proposed for chicken IGF1; (b), location of the mature protein within preproIGF1 and the disulfide bonds (yellow); (c), Amino acid sequence of the mature IGF1; similar colours highlight similar regions within mature IGF1 tertiary structure and amino acid sequence.

Therefore, the biological functions of proteins are governed by the tertiary structure and kinetics between protein-protein interactions associated with signal transduction (Nelson and Cox, 2005). To the best of my knowledge there is no literature on primary, secondary and tertiary structures of IGF1 in guinea fowls elucidated from isolated protein. However, a primary structure similar to cIGF1 has been predicted for guinea fowl preproIGF1 based on computational annotation provided by National Centre for Biotechnological Information, United States National Library of Medicine, National Institute of Health, Maryland, USA (NCBI). Extremely similar primary structures for cIGF1 and gIGF1 are likely to give rise to similar secondary and tertiary structures between the species.

2.4.2. Function of IGF1

IGF1 is one of the key growth factors of the somatotropic axis, a well-coordinated biochemical system that comes to play from embryogenesis (DePablo *et al*., 1990) up to repair of tissues in mature chickens (McMurtry, 1997). Growth promoting activity of IGF1 has been demonstrated in a wide range of tissues including skeletal tissues, skeletal muscles, connective tissues within tendons and nervous tissue (McMurtry, 1998).

Detection of blood borne IGF1 in chick embryos as early as on the sixth day (DePablo *et al*., 1990) suggests direct involvement of IGF1 in very early embryonic development. It's role in the latter part of embryogenesis is suggested by Liu *et al*. (2016) based on mRNA expression profiles from embryonic muscle and liver with

peak values negatively correlated with methylation status at its gene promoter. Due to its role in stimulating cell proliferation and differentiation, IGF1 plays vital roles in tissue regeneration during damage repair in connective tissues of tendons (Chen *et al*., 2008) and retina (Zenlika *et al*., 2012).

IGF1 is a major stimulator in skeletal muscle growth during embryogenesis, post-natal muscle hypertrophy and regeneration (Duclos, 2005). According to Noguchi (2005), IGF1 is the only growth factor involved in both myoblast proliferation and differentiation. Involvement of IGF1 is also evident in mechano-signal transduction of skeletal muscle growth (Tidball, 2005). The functions of avian IGF1 based on literature from experimental evidence reviewed in this chapter are summarized in Figure 3.

IGF1 is involved in stimulating migration and proliferation of Non-astocytic Inner Retinal Glial (NIRG) cells to the retina and hence is involved in development of retina in juveniles and repair of damaged retina in adult chicken (Zenlika *et al*., 2012).

Although IGF1 is undoubtedly a major growth stimulating factor, some reviews suggest more dominant roles for IGF1 in intermediary metabolism than in growth (McMurtry *et al*., 1997). IGF1 is involved in reducing plasma glucose in chicken, a function it shares with insulin (McMurtry *et al*., 1998). However the exact mechanisms involved in hypoglycemic actions of avian IGF1 need further investigation. Goodridge *et al*. (1989) reported a triiodothyronine-induced malic enzyme enhancing activity for IGF1. Considering the function of malic enzyme in synthesis of long chain fatty acids, this suggest that IGF1 favors lipid biosynthesis.

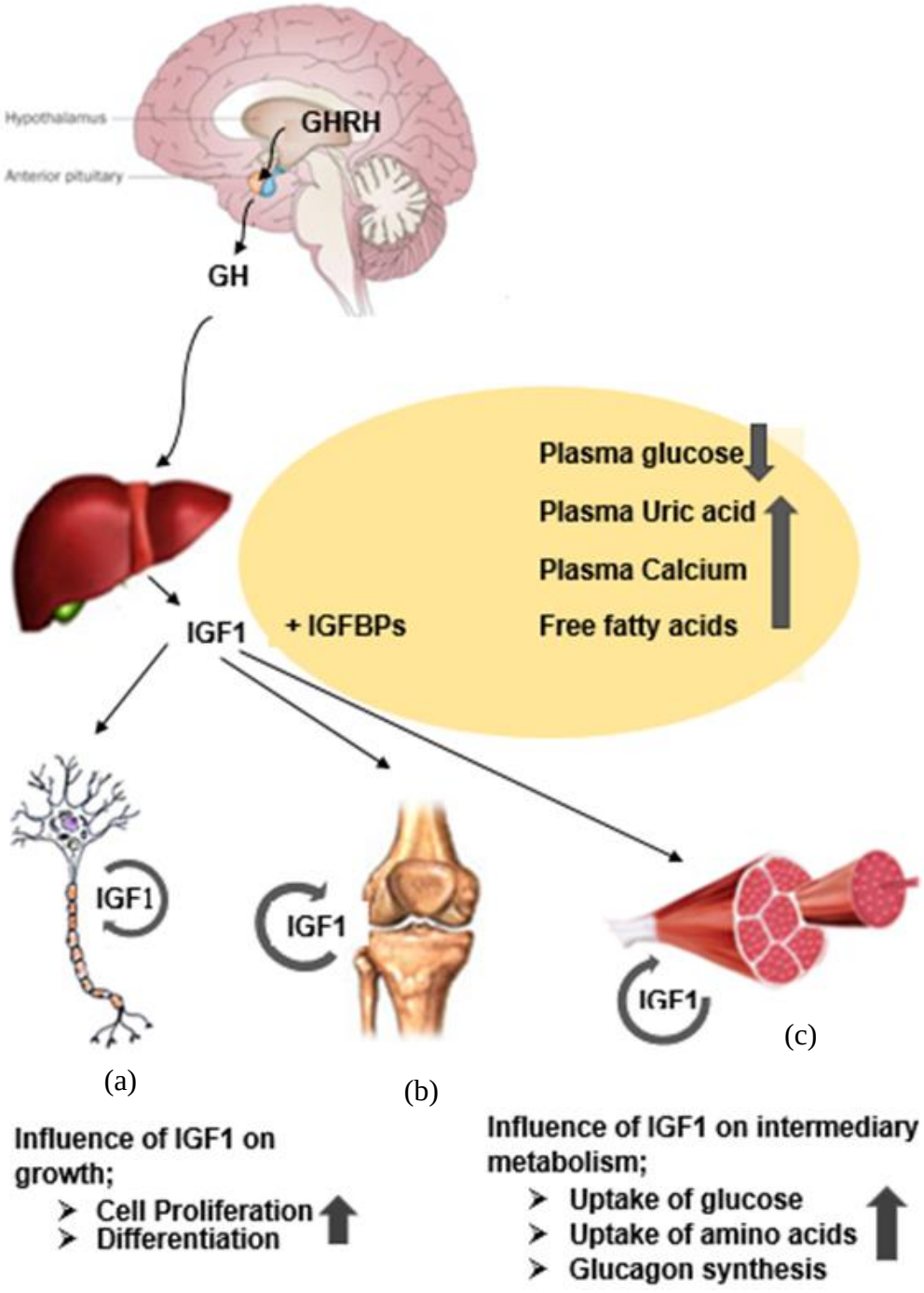


Figure 3. Schematic representation of IGF1 functions in poultry: IGF1 secreted from liver during embryonic, post-hatch and juvenile growth stages has endocrine functions on target tissues such as neurons and neuroglia cells (a), Skeletal bones (b), skeletal muscles (c); IGF1 secreted by IGF1 sensitive tissues also have autocrine and paracrine functions to stimulate growth in GH independent pathways.

2.4.3. Mode of action of IGF1

Most of IGF1 mediated growth regulation occurs via the somatotropic axis (McMurtry *et al*., 1997). IGF1 of hepatic origin travels to IGF1 sensitive tissues in a ternary complex bound to their cognate binding proteins (Tidball, 2005) through blood and exert endocrine functions. However, there is evidence for IGF1 synthesis in response to GH-independent factors (Yu *et al.*, 2015). IGF1 secreted by extrahepatic tissues such as brain, muscles, skeletal tissues have autocrine and/or paracrine growth stimulating effects. In all its functions, signal transduction of IGF1 is initiated by specific binding to Insulin-like growth factor 1 receptor (IGF1R), a member of family of tyrosine kinase receptors that include insulin, IGF1 and IGF2 receptors. Mechanisms of IGF1-mediated downstream signal transduction is less well characterized in birds unlike in mammals. A study by Yu *et al.* (2015) provides strong evidence for IGF1 mediating its downstream signal transduction through PI3K/Akt signaling pathway during myoblast proliferation in chicken embryos. Beside this effect IGF1 also upregulated myogenic factors gene (C-Myc) and inhibition of myostatin gene resulting in cumulative expansion of myoblasts. Although most of IGF1-mediated signal transduction remains to be elucidated, available information to date suggests that IGF1 stimulates growth by increasing amino acid uptake, DNA replication and protein synthesis, terminally stimulating cell proliferation and differentiation (McMurtry *et al*., 1997; Bhattacharya *et al*., 2015).

In mammals, growth regulation mediated by IGF manifests its action through two main signal transduction pathways, namely Mitogen activated protein Kinase (MAPK/ERK1/2) pathway and phosphatidylinositol 3-kinase pathway (PI3K)/ protein

kinase B (Akt) pathway which also contains a complex network of proteins (Yu *et al*., 2015). Terminally, these influence the transcription of proteins involved with cell cycle.

2.4.4. IGF1 gene (*IGF1*)

IGF1 gene is present on chromosome 1 in both chicken (Kajimoto and Rotwein, 1991) (GenBank accession number, NC_006088.4) and guinea fowl (GenBank accession number, NC_034409; Vignal *et al.,* 2017) genomes, while its human counterpart is present on chromosome 12 (GenBank accession number, NC_000012.12). Chicken IGF1 gene (*cIGF1*) is the most studied avian IGF1 gene. It is about 48 kb long and appears more compact than mammalian IGF1 genes and is made up of 4 exons. Exon 1 contains the 5'UTR and a coding region while exon 4 contains a protein coding segment and the 3'UTR. The exons 2 and 3 are protein coding (Kajimoto and Rotwein, 1991). Unlike its most studied chicken homologue, there is no detailed published literature on structural organization of guinea fowl IGF1 gene (*gIGF1*). The genomic sequence of g*IGF1* was only available in June 2017, as part of the whole genome sequence by Vignal *et al.* (2017) (GenBank accession number, NC_034409; GenBank Gene ID, 110393921) together with annotated features of mRNA and coding sequences. There is no gene map available for *gIGF1* in the public domain, therefore, Figure 4 was developed based on the annotations provided by GenBank for the released *gIGF1* genomic sequence during this review.

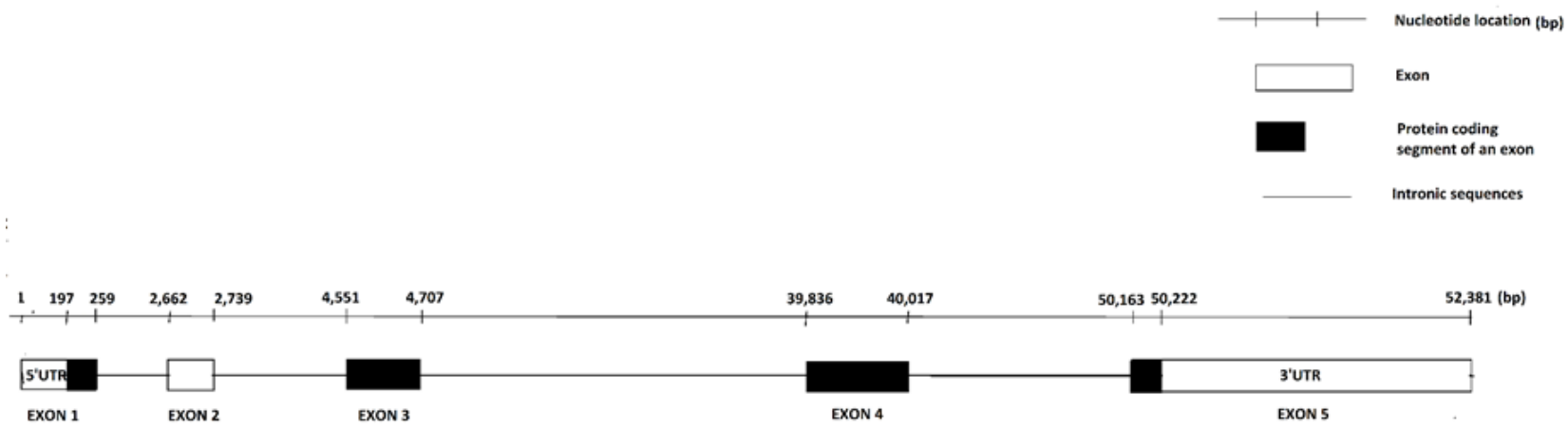


Figure 4. Proposed gene map for guinea fowl *IGF1* (Sequence Data source, Genomic sequence of chromosome 1, accession no., NC_034409; gene ID, 110393921, GenBank genomic database, NCBI)

2.4.4.1. SNPs within avian IGF1 gene and their associations with growth

Nie *et al*. (2005) identified a total of 15 SNPs within *cIGF1* including three SNPs in the 5' UTR, one within an intron and eleven SNPs within the 3'UTR. Three SNPs, including a single SNP each within the translation initiation codon, exon 3 and translation stop codon were reported in a cross between a broiler line and a layer line of chicken (Bian *et al*., 2008). These SNPs were highly associated with body weights at week 4 and 6. A SNP reported within the promoter region first described by Nagarajah *et al*. (2000) has also been reported in other native chicken breeds, including Wanzhai Yellow breeds (Wang *et al*., 2005) and Indian coloured broilers (Pandey *et al*., 2013).

Amills *et al.* (2003) reported a A>C substitution around the TATA box of 5'UTR of *IGF* in Black Penedesenca chicken strains, that was associated with average daily weight gain and feed efficiency at 107th day. Bhattacharya *et al.* (2015) identified 12 haplotypes based on the polymorphisms at 21 loci within exons of *cIGF1* using a control layer, control broiler and Cornish breeds. The SNP loci were distributed in all key functional regions of *cIGF1* including three, two, seven and five SNPs within the 5'UTR, sequences coding for signal peptide, receptor binding domain and Extension (E) domains, respectively. They observed associations between the haplogroups and the body weights in control layer, control broiler and Aseel breeds further providing evidence for dominant role of *IGF1* as a growth and body weight related candidate gene. Ilori *et al*. (2016) also reported polymorphisms within promoter and 5'UTR regions in locally adapted strains of chicken in Nigeria.

Adedibu *et al.* (2013) amplified portions of *IGF1* in guinea fowl and reported some indels, although their positions were not specified with respect to a reference genome. There are no other reports on SNPs in *gIGF1*.

2.5. Insulin-like Growth Factor 2 (IGF2)

Insulin-like growth factor 2 is the second member of the family of growth factors that exhibit significant homology to insulin in structure, receptor binding properties and function. According to the information available to date, it plays significant roles in embryonic growth in aves.

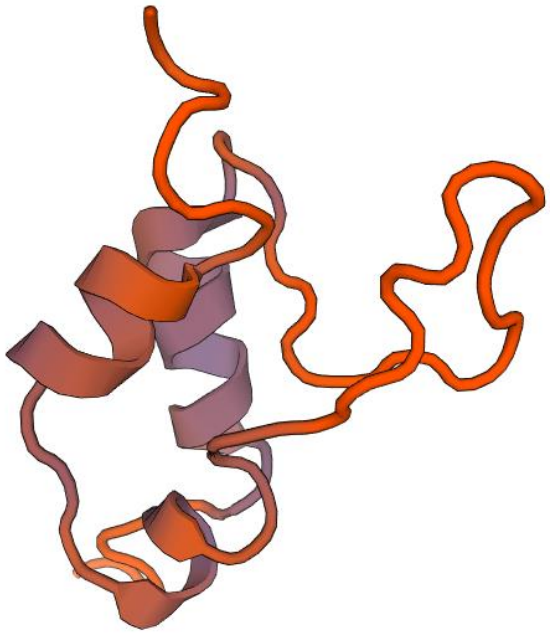
2.5.1. Structure of avian IGF2

Chicken counterpart of preproIGF2 is made up of 187 amino acids weighing 20,837 Daltons (UniProt Consortium, 2017). Kallincos *et al*. (1990) isolated mature IGF2 protein from chicken and determined the complete amino acid sequence which shared high degree of similarities between the amino acids retained in the mature proteins of chicken, rat and humans. The 187 amino acid long preproIGF2 contains a signal peptide from amino acids 1 to 23, mature protein coding region from amino acid no. 24 to 91 and propeptide from residue 92 to 187 (UniProt consortium, 2017). The mature protein is made up of amino terminal B-domain involved in IGF Binding Proteins (IGFBPs), C-domain, A-domain, and the carboxy terminal D domain. Carboxy terminal exhibited greater inter species variation in their amino acid sequences. IGF2 contains binding surfaces to IGFBPs, type I IGF receptors and Type 2 IGF/cation-independent mannose 6-phosphate receptors (Kallincos *et al*., 1990). Due to unavailability of accessible three dimensional structure of IGF2, a three dimensional model developed using SWISS-MODEL workspace version 8.05 (Swiss

Institute of Bioinformatics, Lausanne, Switzerland) (Waterhouse *et al*., 2018) is presented in Figure 5. Published literature on primary structure elucidated from amino acid sequencing of serum purified IGF2 from guinea fowl, secondary or tertiary structures are not available.

2.5.2. Function of IGF2 protein

Functions of IGF2 in poultry is less well understood compared to that of IGF1. A relatively high degree of similarity in the mature proteins and their receptor binding sites between humans and chicken predicts a hormone-like growth factor function for avian IGF2. In mammals IGF2 appears to have a dominant role in embryonic growth. Knock-out mice for *IGF2* attained only 60% of embryonic weight compared to wild type littermates though with no structural deformities (DeChiara *et al*., 1990). IGF2 expression in the eye, limb buds and heart in chick embryo may suggest involvement of IGF2 in morphogenesis in chicken embryo (Engstrom *et al*., 1987). Although Yan *et al*. (2002) suggested growth and carcass related functions for IGF2, experimental evidence for hormone-like growth stimulation by IGF2 in poultry is still unavailable. Increasing abdominal fat deposition in response to intravenous administration of human IGF2 in chicken (Spencer *et al.,* 1996) and observed correlations of IGF2 polymorphism with abdominal fat deposition by Wang *et al*. (2005) suggests roles for IGF2 in lipid metabolism.



(a)

```
Model_01  MCAARQILLLLLAFLAYALDSAAAYGTAETLCGGELVDTLQFVCGDRGFYFSRPVGRNNRRINRGIVEECCFRSCDLALLETYCAKSVKSERDLSATSLAGLPALNKESFQKPSHAKYSKYNVWQ
2129.1.B  ------------------------YRPSETLCGGELVDTLQFVCGDRGFYFSRPA-SRVSRRSRGIVEECCFRSCDLALLETYCATPAKSE--------------------------------

Model_01  KKSSQRLQREVPGILRARRYRWQAEGLQAAEEARAMHRPLISLPSQRPPAPRASPEATGPQE
2129.1.B  ----------------------------------------------------------
```

(b)

Figure 5. Predicted three dimensional structure of chicken IGF2: (a),The three dimensional structure predicted using SWISS-MODEL workspace version 8.05 based on comparisons with human IGF2 as a template (SWISS-MODEL Template Library ID, 2l29.1.B) (b), region within mature IGF2 proteins that show high degree of similarities between chicken IGF2 (Model) and human IGF2 (SWISS-MODEL Template Library ID, 2l29.1.B)

2.5.3. Mechanism of action

IGF2 binds to type 1 tyrosine kinase receptor to initiate IGF2 mediated growth stimulating and metabolic functions. However, IGF2 across taxa also contains a binding domain to type 2 IGF receptor (McMurtry, 1997). Signal transduction mechanisms involved with IGF2 functions post-receptor binding largely remain to be elucidated. Possible pathways predicted by homology with hIGF2 pathways include Mitogen-activated protein kinase (MAPK) cascade, peptidyl-tyrosine phosphorylation, protein kinase B signalling and regulation of histone modification (UniProt consortium, 2017).

2.5.4. IGF2 gene (*IGF2*)

IGF2 is present on the fifth chromosome in chicken (GenBank accession no., NC_006092). Chicken *IGF2* (GenBank gene ID, 395097) is by far the most studied avian *IGF2*. Darling and Brickell (1996) proposed the organization of *cIGF2* gene by aligning genomic DNA and coding sequences detected from cDNA. These findings have been confirmed with the availability of annotated genomic sequence of *cIGF2* from whole genome sequencing of chicken. According to its annotated sequence, *cIGF2* consists of four exons. Transcription of all the four exons gives rise to precursor mRNA of IGF2 isoform X1(GenBank protein ID, XP_015142011.1). The last three exons are transcribed to an alternative transcript (GenBank ID, NM_001030342.1) that is translated to "Insulin-like growth factor 2 precursor". *IGF2* in chicken is reported to be expressed in liver, kidney, heart, and muscle in juvenile chicken biallelecally, with no imprinting (Wang *et al*., 2005).

Unlike its chicken counterpart, *IGF2* gene in guinea fowl (*gIGF2*) has not been isolated and is not well characterized. However, annotated sequence of g*IGF2* that became

available after whole genome sequence of guinea fowl published by Vignal *et al*. (2017) provides some insight into the structural organization of *gIGF2*. *IGF2* in guinea fowl is made up of four coding exons of which all are transcribed to predicted IGF2 transcript variant X1(GenBank accession no., XM_021400964.1) which codes for prepro insulin-like growth factor 2 isoform X1(GenBank protein ID., XP_021256639.1). A second mRNA variant, IGF2 transcript variant X2 is predicted to be translated to a truncated isoform, Insulin-like growth factor 2 isoform X2 (GenBank protein ID., XP_021256640.1). However, no reports are available on actual chemical isolation of these proteins or mRNA and subsequent characterization of *IGF2* in guinea fowls. Therefore, a tentative gene map is proposed based on the computational annotation provided by NCBI in Figure 6.

2.5.5. SNPs within avian IGF2 genes and their associations with body weight and growth traits

Several SNPs have been reported in *cIGF2* across several chicken breeds. Amills *et al*. (2003) described two SNP loci including C>T transition within exon 3 and G>A substitution within intron 2 and found no association with body weight and feeding traits in two divergent strains of Black Penedensca Chicken. Nie *et al*. (2005) reported three SNP loci within introns of *cIGF2*. A single substitution of C>G was reported within exon 2 in a cross between a broiler line and Tauhe Silky chicken by Wang *et al*. (2005). Yang *et al*. (2017) also reported a synonymous mutation in Langshan chicken of China. However, there have been no SNPs previously reported within *gIGF2* in guinea fowls.

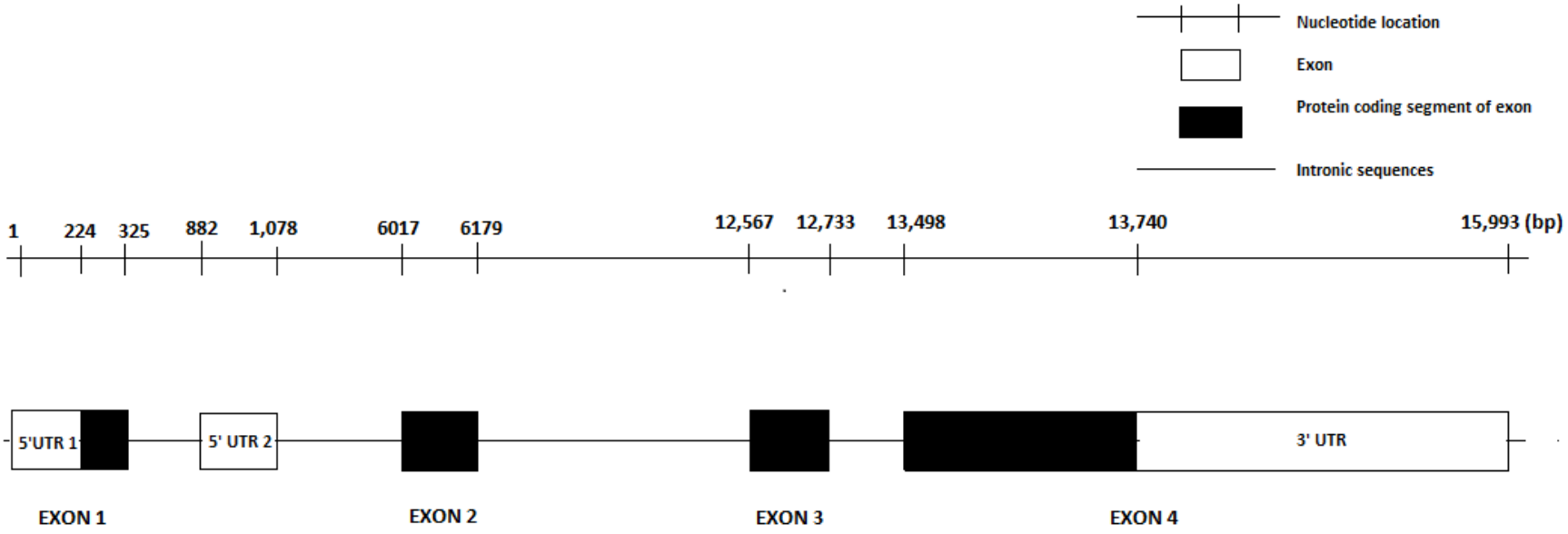


Figure 6. Proposed gene map for guinea fowl *IGF2* (Sequence Data source, Genomic sequence of chromosome 5, accession no., NC_034414; annotation provided by NCBI for GenBank gene ID, 110400777)

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1. Source of experimental animals

3.1.1. Sampling locations and collection of eggs

Eggs from laying helmeted guinea fowls (*Numida meleagris*) were collected from 32 selected guinea fowl growing areas from three main populations of Northern Ghana (TPNG). The three main populations of Northern Ghana included guinea fowl populations from Upper East Region (UER), Former Northern Region (FNR) and Upper West Region (UWR). After the completion of this study FNR was split into three administrative regions namely North East Region, Northern Region and Savannah Region. Therefore it is important to note that FNR currently include three regions. Additionally a fourth population that included birds from a breeder flock maintained at the Animal Research Institute of the Council for Scientific and Industrial Research (CSIR-ARI) was also included in the study making up the four main populations used for early growth performance appraisal. Each of the TPNG was divided into three to four subpopulations to compare guinea fowl populations within the main populations for the purpose of this study. The subpopulations of UER were designated as E1, E2, E3 and E4 while the subpopulations of UWR were designated as W1, W2 and W3. Population from the FNR was subdivided into four subpopulations namely N1, N2, N3 and N4. Locations of the sampling areas that were sampled to obtain subpopulations within UER, FNR and UWR are indicated in Figures 7, 8 and 9, respectively.

Guinea fowl farmers in sample locations were contacted through the regional and district directorates of the Ministry of Food and Agriculture, Ghana and study objectives were explained prior to sample collection. Three different teams carried out collection of eggs simultaneously from the three populations of NG over a period of three days. Guinea fowl

eggs laid within 24 hours from the time of collection were requested and purchased from 5 - 30 guinea fowl farmers per location. The number of eggs collected from a single farmer ranged from 5 to 10 eggs. The collected eggs were labelled according to the farmer, the location, the subpopulation and the main population. The numbers of eggs collected from each location, subpopulation varied depending on the availability of eggs and response of farmers and are summarized in Appendix 1.

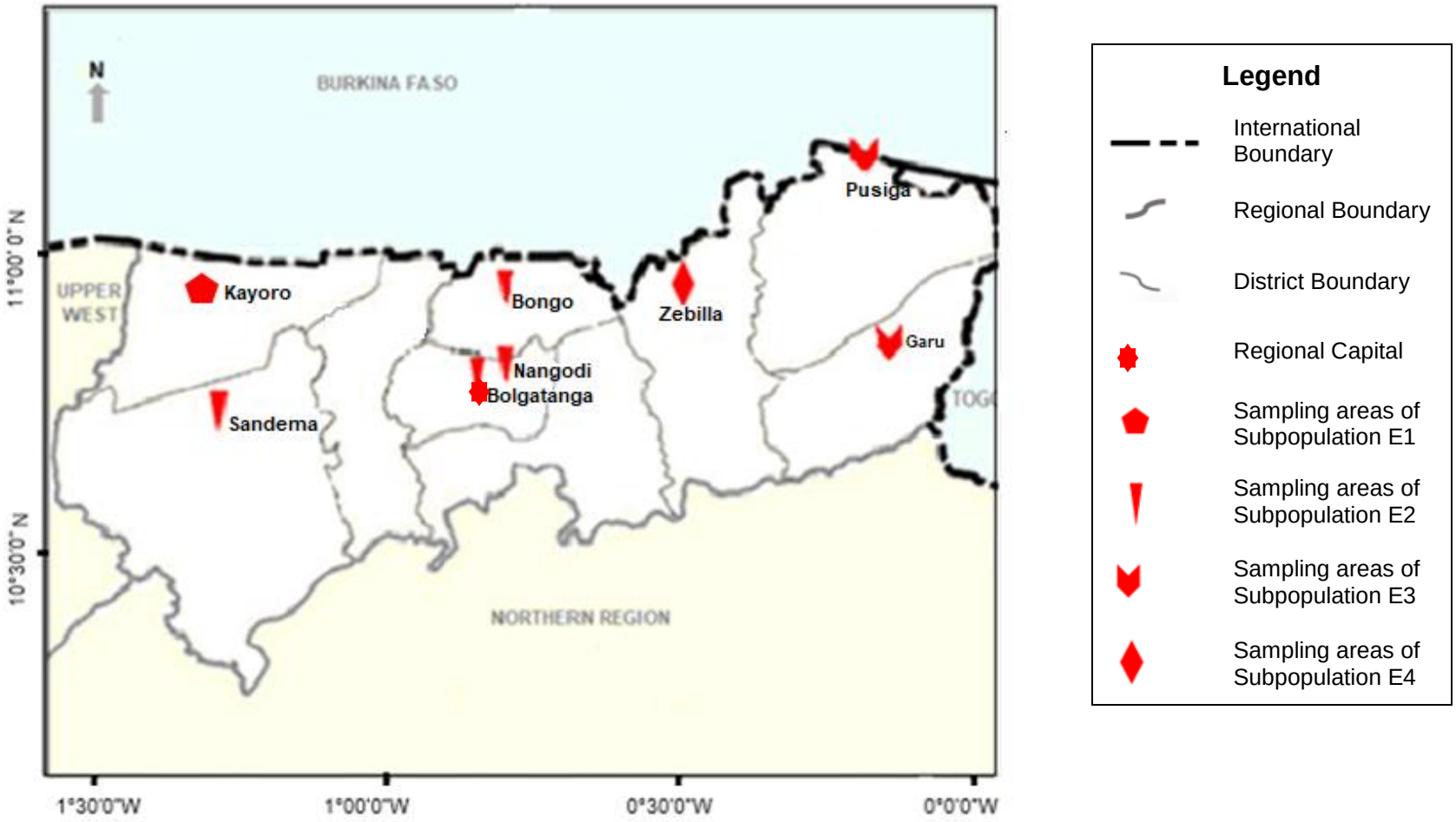


Figure 7. Sampling areas in the Upper East Region

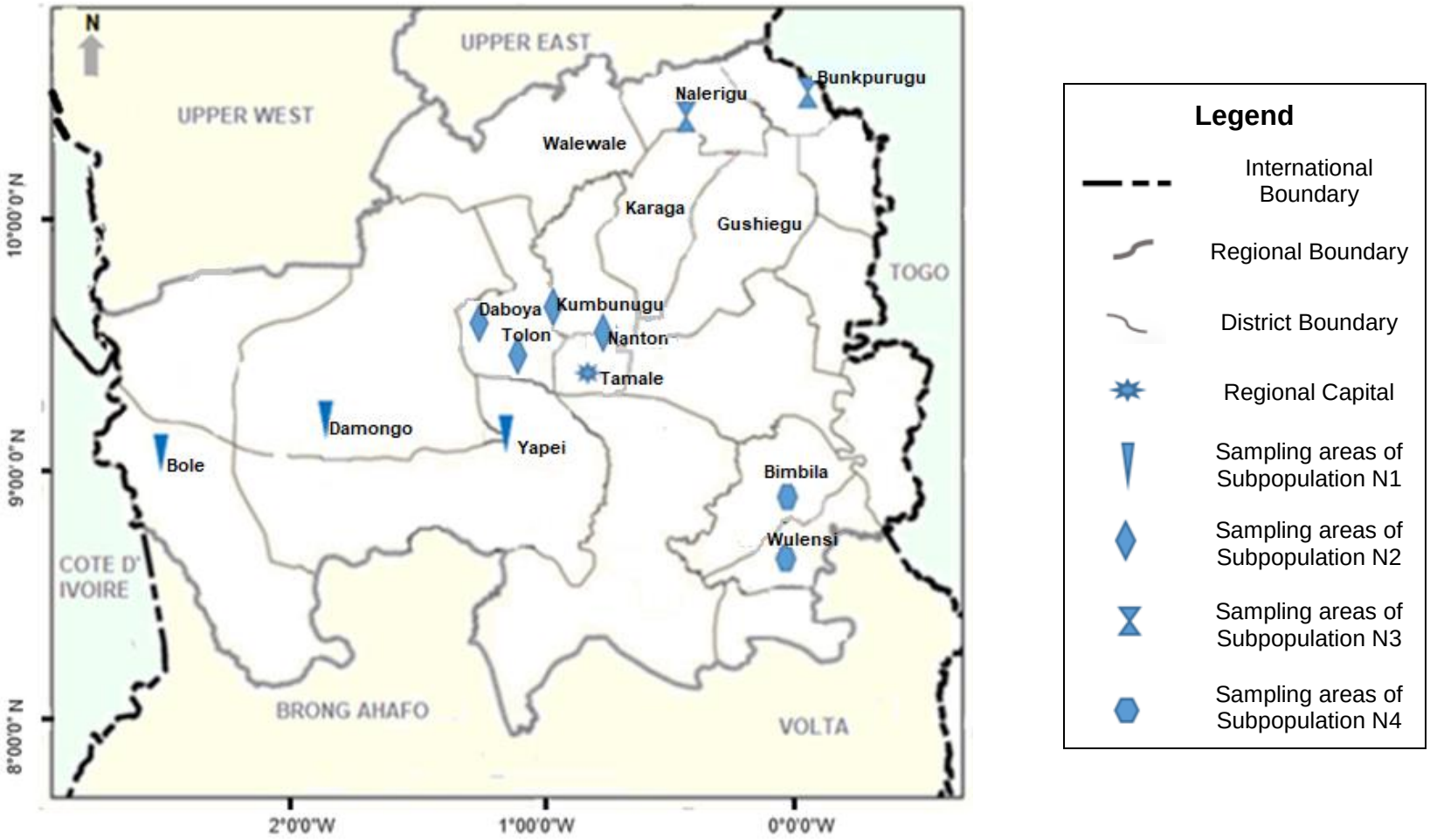


Figure 8. Sampling areas in the Former Northern Region (FNR)

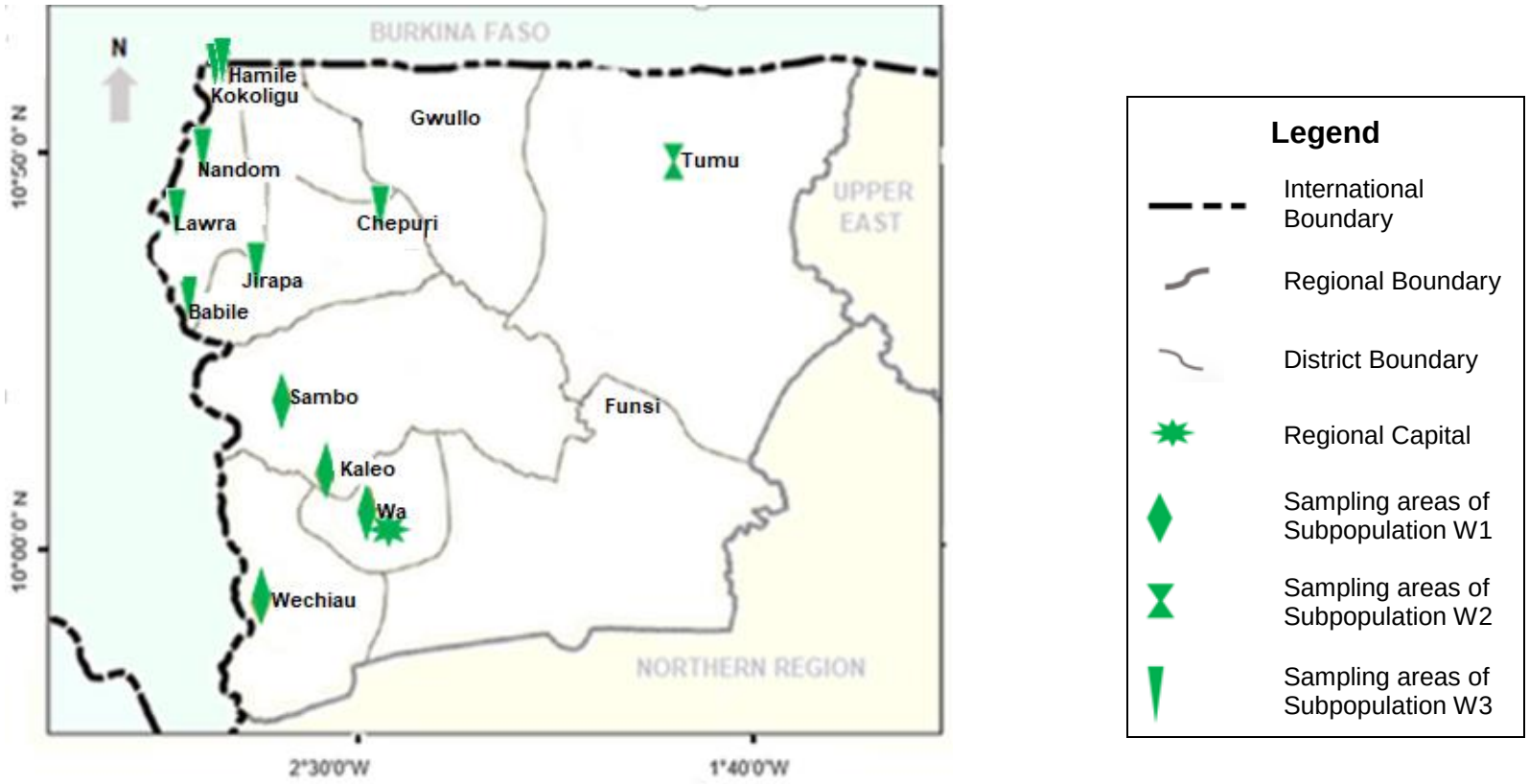


Figure 9. Sampling areas in the Upper West Region

Eggs collected were transported in crates placed in perforated paper cartons minimizing stress on the eggs and assembled at a central location within the regional capitals and stored temporarily for one to two days at a temperature of 16˚C. During storage, eggs were turned back and forth 45˚ from their vertical positions about 90˚ every day and stored pointed ends down. The eggs were then moved to Tamale, from where they were immediately airlifted to Accra and then transported to the Animal Research Institute, Accra, Ghana.

3.1.2. Breeder flock maintained at Animal Research Institute (ARI) of the Council for Scientific and Industrial Research (CSIR)

The breeder flock at ARI was established in 2016 from keets hatched from eggs collected from the same locations in NG except for two additional locations in the Upper West Region that were included in the present study, following similar guidelines during sample collection. Eggs were incubated in an artificial incubator (Hyderabad Incubators, Hyderabad, India) at 37.5˚C and 60% relative humidity for 25 days. Fertile eggs identified on the 25th day by candling were transferred to hatcher compartment maintained at 36.5˚C and 70% relative humidity. The keets hatched around the 28th day were raised up to the eighth week under standard practices of brooding according to Ahiagbe *et al.* (2016) followed by feeding a standard grower diet up to the 12th week. Beyond the 12th week, they were fed with a standard breeder diet and maintained according to recommendations for breeder stock management practices (Ahiagbe *et al*., 2016). Eggs were collected from laying guinea fowl breeders of this breeder flock maintained at the Guinea fowl Resource Centre, CSIR-ARI for four days around the same time when the collection of eggs was on-going in various sample locations of NG.

3.1.3. Incubation of eggs

Upon arrival, all the eggs collected from the TPNG and the CSIR-ARI flocks were stored for 24 hours in a storage room maintained at 18˚C and 70 – 80 % relative humidity. Eggs that were too small, cracked or grossly misshapen were discarded. The eggs were surface-disinfected and incubated at 37.5˚C and 60% relative humidity for 25 days in an artificial incubator (Hyderabad Incubators, Hyderabad, India). Eggs from different locations were incubated in separate setter trays. On the twenty-fifth day, eggs were candled to identify fertile eggs. Infertile eggs or eggs with dead embryos were removed from the setter. Fertile eggs were transferred to the hatcher on the 25th day and hatched in different hatcher trays. The hatcher was maintained at a temperature of 36.5˚C and relative humidity of 70%. Guinea keets that hatched were individually tagged and moved to the brooder house. The numbers of eggs set, fertile eggs, eggs hatched per location, subpopulation and main population are summarized in Appendix 1.

3.2. Appraisal of early growth in guinea fowl keets

Upon arrival at the brooder house, the day old keets were randomly allocated to 6-8 replicates of about 40 keets per main population in Northern Ghana in such a way that the representation of subpopulations were similar across replicates. The keets from CSIR-ARI flock were included as a separate group. All the keets were raised under the same conditions with provision of artificial lighting, heating, a diet formulated for guinea keets and water according to Ahiagbe *et al*. (2016). Biosafety protocols as described by Ahiagbe *et al*. (2016) were adhered to from preparation of brooder house until the end of the eighth week.

At the end of the eighth week all surviving keets were transferred to a deep litter house. Birds per each of three populations were allocated into 2-3 replicates, each consisting of 15-18 birds. Each replicate was housed in a separate compartment. The grower guinea fowls were fed with a formulated grower diet adhering to standard biosafety measures and good production practices.

Throughout the study period, body weights at weekly intervals were recorded for each bird. The feed intake per replicate and mortalities were also recorded daily. Data collected were used to calculate growth rate per individual bird at selected time intervals. Total feed intake per population and FCR were also calculated. Analysis of variance (ANOVA) was performed using R Version 0.99.489 (R Core team, 2016). Means of body weights measured and growth rates calculated at specific time intervals were compared between the three populations and the subpopulations using Tukey-Kramer method (Montgomery, 2013).

3.3. Sample collection for genotyping and sex determination using PCR

3.3.1. Sample collection

Due to high level mortalities, only 112 birds survived from TPNG at the end of growth appraisal. Five milliliters of whole blood was drawn from the wing vein from all surviving birds into EDTA coated sterile vacutainer tubes. DNA was extracted from each blood sample using DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA, USA). Extracted DNA was stored at -20˚C until further use.

3.3.2. Sex determination by PCR

Due to low accuracy of sex determination based on phenotypic methods or vent sexing in guinea fowls, sex of all surviving birds were determined by PCR using methods described by Ahiagbe *et al*. (2017). PCR was performed using two sets of primers including Universal Sex Primer 1 (USP1) (5'CTATGCCTACCACMTTCCCTATTGC3'), Universal Sex Primer 3 (USP3) (5'AGCTGGAYTTCAGWSCATCTTCT3') together with internal control primers namely Forward Control Primer (CPE15F) (AAGCATAGAAACAATGTGGGAC) and Reverse Control Primer (CPE15R) (5'AACTCTGTCTGGAAGGACTT3'). PCR consisted of 0.2 mM each of dNTPs, 2 µM of each target and control primers, 20 ng DNA, 0.25 U Taq Polymerase, 1 x Green Go Taq flexi buffer and 1.5 mM magnesium chloride (GoTaq® PCR Core System I, Promega Corporation, Madison, USA) in a final volume of 20 µl. Amplification of sex specific targets were achieved in a thermal cycler (Bio-Rad C1000 TM Thermal cycler, Bio-Rad Laboratories, Inc. USA) with an initial denaturation step at 95˚C for 10 minutes, followed by 35 cycles at 95˚C for 30 seconds, annealing at 60˚C for 30 seconds, elongation at 72˚C for 60 seconds and final elongation at 72˚C for 5 minutes (Ahiagbe *et al*., 2017).

PCR products were resolved by electrophoresis on a 1.5% agarose gel at 100 V for 30 min in TBE buffer (1 M Tris base, 1M Boric acid, 0.02 M EDTA), stained with ethidium bromide under a UV transilluminator. Birds with double bands were identified as females while those with single bands were identified as males.

3.4. Identification of SNPs in exonic regions of *IGF1* and genotyping

A total of 84 birds out of those that survived (n=112) at the end of early growth appraisal from TPNG including UER (n = 17), FNR (n = 22) and UWR (n = 45) were used for

SNP identification and genotyping of *gIGF1*. The four protein coding exons of *gIGF1*were amplified using primers first described by Bhattacharya *et al*. (2015) using a modified protocol. Purified PCR products were sequenced in both forward and reverse directions. The generated genomic sequences were submitted to GenBank. Genomic sequences generated from direct sequencing were aligned with the reference genomic sequences (GenBank accession no, NC_034409.1; Vignal *et al*., 2017) for *de novo* SNP identification with Nucleotide BLAST using BLASTN 2.7.1+ version of NCBI (National Center for Biotechnology Information, United States National Library of Medicine, National Institutes of Health, Maryland, USA; Zhang *et al.*, 2000) and MEGA ver. 7 (Kumar *et al*., 2016). Haplotypes present among sampled birds of protein coding exons of g*IGF1* were constructed using Arlequin ver. 3.1(Excoffier and Lischer, 2010). The frequencies of SNPs, genotypes at polymorphic loci, haplotypes and haplogroups were determined using GenAlEx software ver. 6.5 (Peakall and Smouse, 2012). Arlequin ver. 3.1 (Excoffier and Lischer, 2010) were used to determine haplotype diversity.

3.5. Identification of SNPs in IGF2 gene

Genomic sequences of *gIGF2* in the 84 birds selected for genotyping g*IGF1* were amplified with primers proposed by Nie *et al.* (2005) and sequenced. SNPs within amplified targets were identified by aligning generated genomic sequences using MEGA ver. 7 (Kumar *et al*., 2016) and Nucleotide BLAST using BLASTN 2.8.0+ version of NCBI (Zhang *et al.*, 2000) with respect to reference guinea fowl sequence (GenBank accession no., NC_034414.1). Unlike in *gIGF1* of which all protein coding exons were covered, enabling construction of haplotypes, haplotypes of g*IGF2* were not constructed due to inadequate gene-wide coverage. Frequencies of allelic variants

and genotypes were calculated using GenAlEx software ver. 6.5 (Peakall and Smouse, 2012).

3.6. Determination of associations among identified SNPs within IGF1 and IGF2 genes, body weight and growth traits during early growth of guinea fowls

The associations between the genotypes at each of the novel SNP identified and traits of interest were estimated using the linear model $Y_{ijkl} = \mu + P_i + S_j + GTSNP_k + e_{ijkl}$ where, Y_{ijkl} is the given dependable variable, μ is the overall mean, P_i is the effect of i^{th} sample Population, S_j is the effect of j^{th} Sex, $GTSNP_k$ is the effect of the k^{th} genotype of a given SNP and e_{ijkl} is the random error. The traits used as dependable variables included the body weights measured at first, second, third, fourth, fifth, sixth, seventh, ninth and eleventh weeks, growth rates measured at specific weekly intervals of 1-2, 2-3, 3-4, 6-7, 9-11 and the overall growth rate. Additionally the effect of haplogroup on the selected phenotypic traits was also estimated using the linear model $Y_{ijkl} = \mu + P_i + S_j + HPG_k + e_{ijkl}$ where, Y_{ijkl} is the given dependable variable, μ is the overall mean, P_i is the effect of i^{th} sample Population, S_j is the effect of j^{th} Sex, HPG_k is the effect of the k^{th} haplogroup and e_{ijkl} is the random error.

CHAPTER 4

COMPARISON OF EARLY GROWTH AND SURVIVABILITY IN THREE INDEGINOUS GUINEA FOWL POPULATIONS OF NORTHERN GHANA

4.1. Summary

This study compared three guinea fowl populations from NG in terms of their Body Weights (BWs), Growth Rates (GRs) and survivability during the first eleven weeks of life. Eggs collected from TPNG together some collected from an experimental flock maintained at Animal Research Institute (ARI flock) were hatched. The keets were all raised under best practices for brooding during the first eight weeks and then up to twelve weeks at the Guinea Fowl Resource Centre, ARI, Accra. Weekly BWs from week one to eleven, daily feed intake and daily mortalities were recorded in Microsoft Excel spreadsheets and Feed Conversion Ratio (FCR), weekly percentage mortalities were calculated. Data were analysed using R Version 0.99.489 (R Core team, 2016).

Keets from ARI flock were significantly heavier ($p < 0.05$) than those from the TPNG during the first, second and the fourth week. Thereon, BWs were similar among the TPNG and ARI flock. In contrast, among the subpopulations significant differences emerged in BWs from the second week and were more pronounced from the sixth week.

Growth rates measured as weekly weight gains also differed significantly among sub-populations beyond the sixth week although differences in GRs were not significantly different among TPNG. Therefore, although the variations in BWs and GRs were limited among the TPNG, there existed significant variations among subpopulations, creating opportunities to establish genetically divergent populations for growth rate and improve early GRs and BWs in local guinea fowls by selection. Although ARI flock was comparable in GRs with the birds from TPNG, probably due to common descent, very low keet mortalities observed in ARI group suggest survivability of guinea keets can be greatly improved by integrating good breeder stock management practices with post hatch brooding to overcome the overriding challenge of keet mortalities in Northern Ghana and other tropical African countries.

4.2. Introduction

The helmeted guinea fowl (*Numida meleagris*) is one of the important animal genetic resources of the African continent. It is thought to have originated from the Coast of Guinea (Newbold, 1926), an area that spans the coastal zone of present day West Africa. Europeans took this bird to Europe and the Americas where it was domesticated under semi-intensive and intensive systems (Moreki and Radikara, 2013). In some European countries and the United States of America (USA), guinea fowl production has been improved to large scale commercial operations (Nahashon *et al*., 2006) with integrated improvements in nutrition and husbandry coupled with genetic improvement by selective breeding.

They are raised mainly under semi-intensive or extensive system with minimal farm inputs in many African countries including Botswana (Moreki and Seabo, 2012), Benin (Dahouda *et al*., 2007), Nigeria (Ogah, 2013), Zimbabwe (Kusina *et al*., 2012) Ghana (Avornyo *et al*., 2016) and others (Moreki, and Radikara, 2013). However, due to its inherent resistance to most poultry diseases beyond the keet stage, remarkable adaptation to low input production systems and local weather conditions, resource-poor guinea fowl farmers in Africa largely depend on local varieties of this fowl (Moreki, and Radikara, 2013).

In Ghana, guinea fowl is the most common poultry species in northern part of the country (Agbolosu *et al.*, 2012b), where almost every rural household keeps a few birds in their backyard (Dei and Karbo, 2004). Guinea fowl meat and eggs provide important sources of protein in the diet of rural folk in NG. Issaka and Yeboah (2016) indicated that farmers in NG rear guinea fowls profitably mainly for cash returns to meet their daily needs and for important sociocultural roles.

The majority of the guinea fowl farmers (98%) in NG depend solely on local varieties of guinea fowls and rearing specialized exotic breeds improved for meat is negligible (FAO, 2014; Avornyo *et al*., 2016). However, these rich genetic resources are not well characterized. Published literature on important production characteristics including body weight, growth rate and disease resistance of local Ghanaian guinea fowl varieties is scanty. There have been no reports on breeding programmes for genetic improvement of these varieties or phenotypic recording schemes to facilitate initiation of long term breeding programmes for local guinea fowls in Ghana

However, available evidence suggests existence of diversity among the local varieties kept by local farmers in NG (Kayang *et al*., 2010) providing opportunities for future breeding programmes. Agbolosu *et al*. (2012a) observed significant differences in body weights of local guinea fowls originating from Upper West Region (UWR), Upper East Region (UER) and Former Northern Region (FNR) of Ghana during the eight to eighteen weeks growth period. They further suggested that birds from Upper East recorded higher body weights and were suitable for breeding programmes aiming at improving meat traits. By contrast, birds from UWR had smaller bodies, but exhibited superior laying traits in terms of average number of eggs laid during the study period (Agbolosu *et al*., 2012b).

While similar comparative performance trials are necessary to validate these claims, there is still a paucity of published literature on variation of body weight and growth rate traits during the brooding period that include the first eight weeks in local guinea fowls from Northern Ghana. In fact, slow growth rate and limited baseline data to facilitate genetic improvement programmes have been identified as two of the key challenges to upscale local guinea fowl production in tropical African countries (Oke *et al.*, 2004). Farmers in some African countries, however, identified faster growth

among the most preferred traits to be improved in their local poultry breeds (Okeno *et al*., 2011).

As market value of poultry meat is directly determined by the weight of the dressed carcass or the live bird, improving growth rate and overall body weight are among key breeding objectives considered in most poultry breeding programmes (ALBC, 2007). Most long term breeding programmes aiming at selecting divergent lines for faster growth in chicken have used body weights and growth rates during the juvenile stage as selection criteria to achieve genetic gain (Flisar *et al*., 2014). Body weight at eight weeks of age has been the most commonly used selection criterion in these breeding experiments for chicken (Dunnington and Siegel, 1996; Terčič, and Holcman, 2008).

Due to the persistent challenge of high keet mortality during the first eight weeks of growth, several studies have compared the performance of local guinea keets under extensive system and intensive system (Dei *et al*., 2009; Naandam and Issah, 2012; Mohammed and Dei, 2017). However, extensive studies to compare the performance of guinea fowl varieties raised in NG during post hatch and juvenile growth period is limited.

Although growth remains a priority trait in poultry breeding in all economic species, other characteristics pertaining to fertility and survivability should not be overlooked during breed improvement (ALBC, 2007). While high survivability is an important production trait to be improved or maintained when aiming for genetic gains in growth rate, in most breeding programmes, it is even more important for breeds meant for dissemination to resource poor poultry farmers with limited access to veterinary care as those in the northern part of Ghana.

Therefore, this study seeks to characterize and compare three populations of guinea fowls from NG in terms of their body weight, growth rate and survivability during the early growth period. Such information will be of immense importance for researches who plan to design and implement sustainable breeding programmes to facilitate sustainable utilization of this less well studied animal genetic resource.

4.3. Materials and Methods

4.3.1. Animals and their management

In total, 865 day old keets from the four main study populations including UER (n = 250), FNR (n = 242), UWR (n = 322) and an experimental breeder stock maintained at CSIR-ARI (n = 51) were used for the study. For the purpose of this study UER was divided into subpopulations designated as E1, E2, E3 and E4 (Figure 7: Chapter 3) while population from FNR was divided to subpopulations N1, N2, N3 and N4 (Figure 8: Chapter 3). UWR was divided to three subpopulations designated as W1, W2 and W3 (Figure 9: Chapter 3). Guinea fowl growing communities in each subpopulation from where the keets used in this study originated are summarized in Appendix 1. All keets were hatched under identical incubation conditions set within a single batch at the Hatchery Unit of CSIR-ARI from the eggs collected from their respective origins.

Day old keets from each population were randomly assigned to replicates of 40-50 birds with nearly an equal number of keets from a subpopulation represented in all replicates for a given population out of the TPNG. Therefore, both UER and FNR had six replicates each, while UWR had eight replicates. The population from CSIR-ARI (ARI flock; ARI population) was included in one replicate of the total 21 replicates. All keets were individually tagged within the first 24 hours after hatching to minimise stress and trauma during handling.

4.3.2. Management of experimental flocks

Keets belonging to all replicates were raised at the brooder house of the Guinea fowl Resource Centre, Animal Research Institute, Accra, Ghana (Plate 2). Replicates were housed within compartments each measuring 1 m x 1.5 m x 2.5 m for length, breadth and height, respectively. The brooder pens were preheated 24 hours before receiving

the keets. Keets were then brooded with provision of artificial light and heat (Plate 3) tightly regulating the temperatures at 35, 33, 31 and 29°C from first to fourth week, respectively, with the help of gas brooders. Beyond the fourth week, internal temperature of the brooder house was maintained at 29°C up to eight weeks. Best practices for preparing the brooder house and biosafety during daily operations were adhered to according to procedures of Ahiagbe *et al*. (2016) during the entire brooding period of eight weeks. A vaccination schedule was followed according to recommendations by Ahiagbe *et al*. (2016). From day one up to eight weeks, keets were fed with a formulated diet containing 24% Crude Protein (CP) and 12.5 MJ Metabolizable Energy (ME)/kg (Table 1). Feed and water were available *ad libitum*.

At eight weeks surviving birds (n =222) were transferred to a deep litter house, where they were randomly reassigned to replicates consisting of 15-18 birds. In all, UER (n= 34), FNR (n=64), UWR (n=73) and ARI (n=51) populations had 2, 4, 4 and 3 replicates, respectively. Each replicate was housed in a compartment at a stocking density of twenty growers per square meter. From eight weeks to twelve weeks birds were fed with a formulated grower diet with 16.37 % CP and 11.23 MJ ME/kg (Table 1). Feed and water were available *ad libitum*. A prophylactic health management plan for raising growers was followed ensuring biosafety during daily operations. In the 12th week, guinea fowl growers were distributed to guinea fowl farmers as per the requirements of the associated research and development project.

Plate 2. Brooder facility of Guinea fowl Resource Centre, CSIR-Animal Research Institute

Plate 3. Post hatch brooding of guinea fowl keets during the study at the brooder facility of Guinea Fowl Resource Centre, CSIR-Animal Research Institute, Accra

Table 1. Composition of diets fed to guinea fowls at different stages

Ingredients (%)	**0-8 weeks**	**9-12 Weeks**	**Guinea fowl Breeders**
Maize	62.30	55.00	60.00
Soybean meal	21.00	15.00	17.70
Wheat bran	-	14.00	5.00
Di-calcium phosphate	0.70	0.90	0.90
Limestone	0.90	2.00	8.40
Salt (NaCl)	0.15	0.20	0.25
Lysine	0.15	0.20	0.20
Methionine	0.15	0.10	0.10
Fishmeal	13.20	1.00	3.00
Vitamin and mineral premix*	0.25	0.25	0.25
Palm oil	1.20	1.35	2.20
Palm Kernel Cake (PKC)	-	10.00	2.00
Total	**100**	**100**	**100**
Calculated composition			
Metabolizable energy (MJ/kg)	12.50	11.23	11.60
Crude protein (%)	24.00	16.37	16.92
Lysine (%)	1.39	0.80	0.92
Methionine (%)	0.57	0.30	0.35
Crude fiber (%)	2.42	4.54	2.82
Crude fat (%)	3.70	5.02	5.29
Calcium (%)	1.31	1.06	3.40
Available Phosphorus (%)	0.47	0.37	0.36
**P: E	19.20	14.58	14.59

*Vitamin and mineral premix per 100 kg diet: Vitamins, Vitamin A ($8x10^5$ I.U); Vitamin D3 ($1.5x10^4$ I.U); Vitamin E (250 mg); Vitamin K (100 mg); Vitamin B2 ($2x10^2$ mg); Vitamin B12 (0.5 mg); Folic acid (50 mg); Nicotinic acid ($8x10^2$ mg); Calcium panthotenate (200 mg); Choline ($5x10^3$ mg); Trace elements, Mg ($5x10^3$ mg); Zn ($4x10^3$ mg); Cu ($4.5x10^2$ mg); Co (10 mg); I (100 mg); Se (10 mg); Antioxidants, Butylatedhydroxytoluene ($1x10^3$ mg). Carrier: Calcium carbonate q.s.p (0.25 kg),
** P: E - Protein: energy (g protein/MJ ME)

4.3.3. Data collection

Body weight of each bird was recorded using an electronic balance at week 1 (BW1), week 2 (BW2), week 3 (BW3), week 4 (BW4), week 6 (BW6), week 7 (BW7), week 9 (BW9) and week 11 (BW11). Weight gain per week was used as an indicator of growth rate for selected time intervals. Recorded body weights were used to determine growth rates (Formula 1) between weeks 1 and 2 (GR1), weeks 2 and 3 (GR2), weeks 3 and 4 (GR3), weeks 4 and 6 (GR4), weeks 6 and 7 (GR5), weeks 7 and 9 (GR6), weeks 9 and 11 (GR7) and the overall growth rate between week 1 and 11 (GRO). The body weights in between the specified weeks could not be measured due to unavailability of electricity at the experimental pens on those days. Feed intake per replicate was recorded daily and was used to calculate mean feed intake for the entire study period per region. Feed Conversion Ratio (FCR) was calculated using Formula 2. Mortalities were recorded daily and used to calculate Percentage mortalities per week according to Yassin *et al.* (2008) using Formula 3.

$$\text{Growth rate measured as weekly weight gain} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Time interval (weeks)}} \quad (1)$$

$$\text{Feed Conversion ratio (FCR)} = \frac{\text{Total feed consumed per bird (g/bird)}}{\text{Total weight gain per bird (g/bird)}} \quad (2)$$

$$\text{Percentage mortality at week N} = \frac{\text{No. of mortalities during week N}}{\text{No. of keets at day 1}} \times 100\ \% \quad (3)$$

4.3.4. Data analysis

Data collected were entered into Microsoft Excel spreadsheets and validated. Analysis of variance (ANOVA) was performed for weekly body weights and growth rates at specified time intervals using the generalized linear model below.

$$Y_{ijk} = \mu + P_i + S_j + e_{ijk}$$

where, Y_{ijk} is the given dependable variable, μ is the overall mean, P_i is the effect of i^{th} sample Population, S_j is the effect of j^{th} subpopulation and e_{ijk} is the random error. The traits used as dependable variables included the body weights measured at first, second, third, fourth, fifth, sixth, seventh, ninth and eleventh weeks, growth rates measured at specific weekly intervals of 1-2, 2-3, 3-4, 6-7, 9-11 and the overall growth rate. Least Square Means (LSM) and Standard Error (SE) were calculated. Multiple pairwise comparisons were done using Tukey's method. All statistical analysis was performed using R Version 0.99.489 (R Core team, 2016).

4.4. Results

4.4.1. Body weight traits

The mean body weights of the four main guinea fowl populations including those from the TPNG and ARI flock measured at various ages during their early growth performance appraisal are presented in Table 2. Body weights at week one did not vary significantly ($p > 0.05$) among the TPNG. However all TPNG differed significantly from the offspring of the experimental breeder flock of CSIR-ARI at week one. At week two mean body weights between Upper East and ARI flock were comparable but differed from the other populations. Pairwise comparisons of weekly body weights were significant between some populations from the first to fourth week. However, the differences in body weights by population were not statistically significant ($p > 0.05$) beyond the sixth week.

Table 3 shows the weekly mean body weights of keets from the different subpopulations within each of TPNG. The mean body weights at week one were not different when compared among the subpopulations within TPNG but with some subpopulations significantly differing from that of the ARI flock ($p < 0.05$). Contrary to the TPNG, body weights of birds of subpopulations differed significantly beyond the sixth week with subpopulations demonstrating more pronounced effects on body weights ($p < 0.01$) beyond 6 weeks. All keets in subpopulation E1 of the UER died in the first week and so there was no data for that subpopulation.

Table 2. Mean body weights (g) of local guinea fowls from three populations of Northern Ghana and ARI flock at various ages

Trait		UER	FNR	UWR	CSIR-ARI
BW1	LS Mean	$37.40^{b} \pm 1.61(131)^{\S}$	$34.89^{b} \pm 1.43(165)$	$35.09^{b} \pm 1.23(223)$	$46.36^{a} \pm 2.60(50)$
	Range	34.24 - 40.56	32.07 - 37.70	32.67 - 37.52	41.25 - 51.48
BW2	LS Mean	$46.92^{a} \pm 0.89(111)$	$43.39^{b} \pm 0.81(135)$	$43.05^{b} \pm 0.69(184)$	$47.98^{a} \pm 1.34(49)$
	Range	45.17 - 48.67	41.80 - 44.97	41.69 - 44.41	45.35 - 50.61
BW3	LS Mean	$64.95^{a} \pm 1.82(84)$	$62.57^{ab} \pm 1.57 (114)$	$58.25^{b} \pm 1.34(155)$	$63.23^{ab} \pm 2.41(49)$
	Range	61.36 - 68.54	59.49 - 65.65	55.61 - 60.90	58.48 - 67.98
BW4	LS Mean	$81.84^{ab} \pm 3.05 (66)$	$78.76^{ab} \pm 2.44 (103)$	$72.76^{b} \pm 2.12(137)$	$84.41^{a} \pm 3.57(49)$
	Range	75.85 - 87.83	73.97 - 83.56	68.60 - 76.92	77.38 - 91.44
BW6	LS Mean	$134.16^{a} \pm 6.99 (49)$	$128.85^{a} \pm 5.34(84)$	$127.81^{a} \pm 4.85(102)$	$140.23^{a} \pm 7.22(48)$
	Range	120.39 - 147.93	118.33 - 139.37	118.26 - 137.36	126.01 - 154.44
BW7	LS Mean	$181.38^{a} \pm 10.18(42)$	$180.59^{a} \pm 7.78(72)$	$174.59^{a} \pm 7.33(81)$	$191.65^{a} \pm 9.84(47)$
	Range	161.31 - 201.44	165.27 - 195.92	160.14 - 189.04	172.27 - 211.04
BW9	LS Mean	$270.66^{a} \pm 15.38(34)$	$257.56^{a} \pm 11.21(64)$	$262.96^{a} \pm 10.29(73)$	$288.03^{a} \pm 13.52(46)$
	Range	240.33 - 300.98	235.46 - 279.66	242.68 - 283.24	261.37 - 314.68
BW11	LS Mean	$369.05^{a} \pm 19.44(33)$	$353.52^{a} \pm 14.07(63)$	$367.39^{a} \pm 13.65(67)$	$384.09^{a} \pm 16.84(46)$
	Range	330.71 - 407.39	325.77 - 381.27	340.48 - 394.30	350.89 - 417.29

BWn, Body weight at week **n**, **n** =1,2,3,4,6,7,9,11; Means of populations within a row with different superscripts differ significantly ($p < 0.05$); ^{NS} Comparisons among the means of populations are not statistically significant at 95% confidence level; § Numbers within parenthesis represent the number of observations

Table 3. Comparison of mean body weights (g) of local guinea fowls at different ages by subpopulations in the TPNG and ARI flock

Subpopulation/ Pop.	BW1	BW2	BW3	BW4	BW6	BW7	BW9	BW11
E2	38.00^{ab} ± 1.94 (91)	47.56^{a} ± 1.06 (77)	67.42^{ac} ± 2.26 (53)	85.53^{ab} ± 3.86 (38)	136.29^{bc} ± 9.41 (23)	185.15^{b} ± 14.73 (17)	266.71^{bc} ± 24.31 (12)	341.53^{b} ± 31.63^{b} (11)
E3	36.52^{ab} ± 8.28 (5)	52.47^{abc} ± 5.37 (3)	80.33^{abc} ± 9.49 (3)	110.20^{abc} ± 23.82 (1)	180.80^{abc} ± 45.15 (1)	294.90^{ab} ± 60.74 (1)	386.40^{abc} ± 84.22 (1)	459.60^{ab} ± 104.91 (1)
E4	35.96^{ab} ± 3.13 (35)	44.79^{abc} ± 1.67 (31)	58.63^{abc} ± 3.11 (28)	75.59^{abc} ± 4.58 (27)	130.34^{bc} ± 9.03 (25)	173.98^{b} ± 12.40 (24)	267.40^{bc} ± 18.38 (21)	379.15^{b} ± 22.89 (21)
N1	34.28^{b} ± 2.57 (52)	40.80^{bc} ± 1.55 (36)	57.88^{bc}± 3.11 (28)	67.03^{bc} ± 4.58 (27)	106.19^{c} ± 9.85 (21)	157.13^{b} ± 16.23 (14)	230.04^{bc} ± 22.51 (14)	323.15^{b} ± 28.04 (14)
N2	34.79^{b} ± 2.35 (62)	44.56^{abc}± 1.25 (55)	61.56^{abc} ± 2.32 (50)	76.53^{bc} ± 3.59 (44)	114.52^{c} ± 7.52 (36)	156.20^{b} ± 10.27 (35)	240.02^{bc} ± 14.89 (32)	333.34^{b} ± 18.84 (31)
N3	35.63^{ab} ± 3.50 (28)	41.74^{abc} ± 1.86 (25)	62.32^{abc} ± 3.59 (21)	80.55^{bc} ± 5.46 (19)	133.24^{bc} ± 11.66 (15)	189.34^{b} ± 17.53 (12)	253.99^{bc} ± 25.39 (11)	329.97^{b} ± 31.63 (11)
N4	35.61^{ab} ± 3.86 (23)	47.08^{abc} ± 2 .13 (19)	74.83^{a} ± 4.24 (15)	108.11^{a} ± 6.61 (13)	206.03^{a} ± 13.03 (12)	278.50^{a} ± 18.31 (11)	398.41^{a} ± 31.83 (7)	540.63^{a} ± 39.65 (7)
W1	36.12^{ab} ± 1.95 (90)	44.11^{abc} ± 1.08 (74)	58.29^{bc} ± 2.10 (61)	72.84^{bc} ± 3.18 (56)	129.16^{c} ± 6.81 (44)	173.87^{b} ± 9.60 (40)	266.52^{bc} ± 14.66 (33)	368.56^{b} ± 20.19 (27)
W2	36.38^{ab} ± 4.14 (20)	44.92^{abc} ± 2.26 (17)	64.06^{abc} ± 3.99 (17)	83.48^{abc} ± 5.96 (16)	160.79^{ab} ± 12.07 (14)	217.46^{b} ± 16.85 (13)	329.29^{ab} ± 24.31 (12)	434.95^{ab} ± 30.29 (12)
W3	34.05^{b} ± 1.74 (113)	41.87^{c} ± 0.96 (93)	56.95^{b} ± 1.87 (77)	70.06^{c} ± 2.95 (65)	115.97^{c} ± 6.81 (44)	155.71^{b} ± 11.48 (28)	233.49^{c} ± 15.13 (28)	337.31^{b} ± 19.82 (28)
ARI	46.34^{a} ± 2.62 (50)	47.98^{a} ± 1.33 (49)	63.23^{abc} ± 2.37 (48)	84.41^{abc} ± 3.44 (48)	140.23^{bc} ± 6.66 (47)	191.65^{b} ± 9.05 (45)	288.03^{bc} ± 12.70 (44)	384.09^{b} ± 15.82 (44)

Pop., Population**; BWn**, Body weight at week **n**, **n** = 1, 2, 3, 4, 6, 7, 9, 11; **E2, E3, E4**, Subpopulations in the Upper East Region; **N1, N2, N3, N4**, Subpopulations of former Northern Region; **W1,W2,W3,** Subpopulations of Upper West Region; Means of subpopulations within a column with different superscripts differ significantly ($p < 0.05$)

4.4.2. Growth curves of guinea fowls from three populations of Northern Ghana and their subpopulations

The growth curves for guinea fowls from TPNG and CSIR-ARI from week one to week eleven are given in Figure 10. Growth curves for the guinea fowls from all the eleven subpopulations are compared in Figure 11 while growth curves for each of the subpopulations in UER, FNR and UWR with CSIR-ARI flock are presented for comparisons within main populations in Figures 12, 13, and 14, respectively.

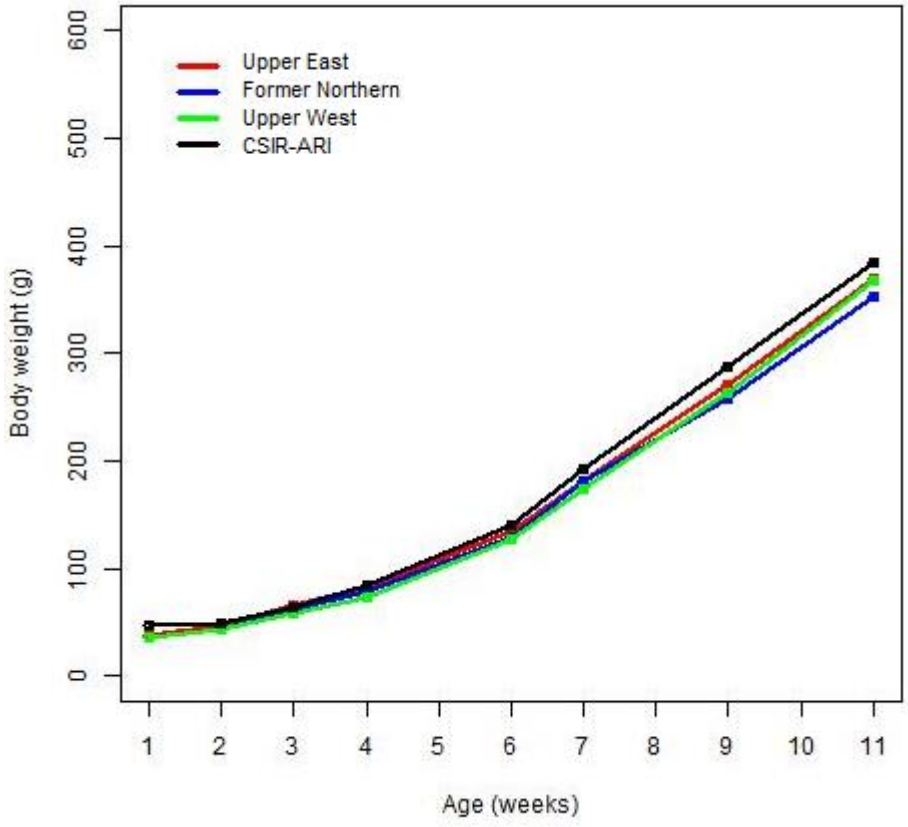


Figure 10. Growth curves for guinea fowls from Upper East, former Northern and Upper West regions and the breeder flock at CSIR-ARI during the early growth stage (0 -11 weeks)

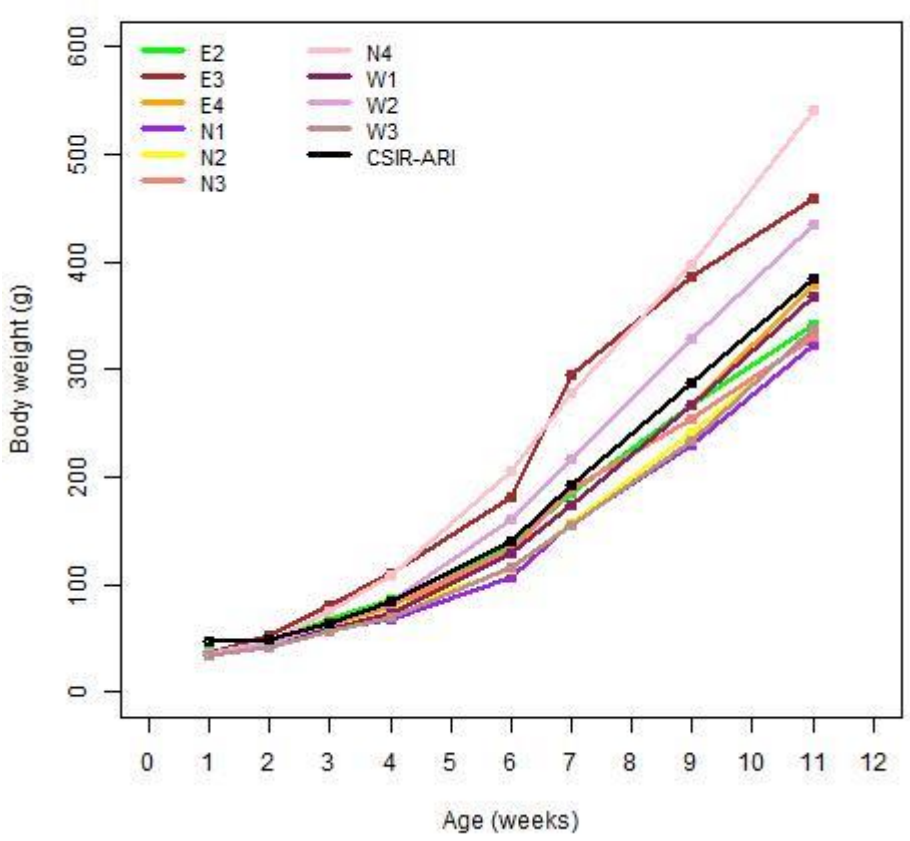


Figure 11. Growth curves for guinea fowls from designated subpopulations within Upper East, former Northern and Upper West regions and ARI flock during the early growth stage (0 -11 weeks)

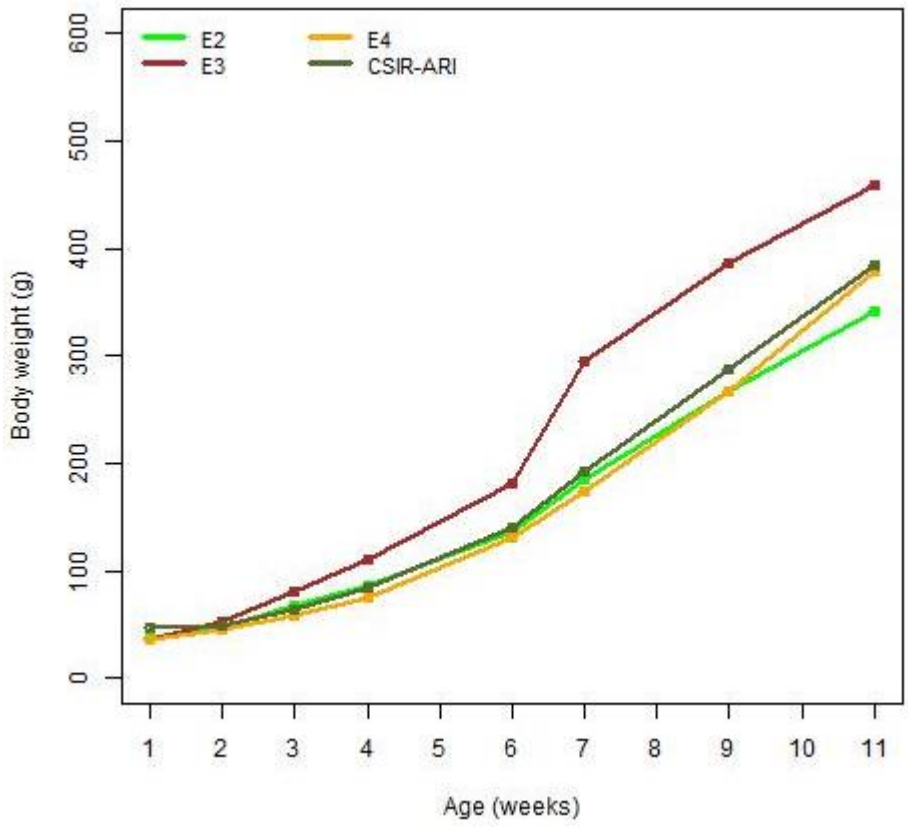


Figure 12. Growth curves for guinea fowls from subpopulations within the Upper East Region and CSIR-ARI flock

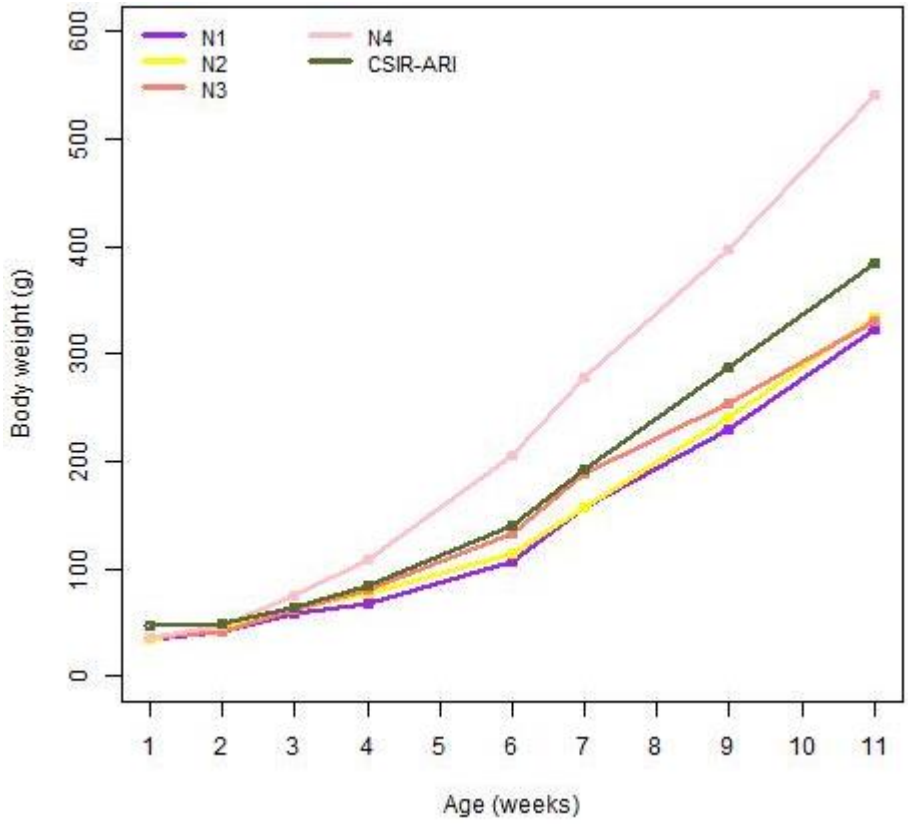


Figure 13. Growth curves for guinea fowls from subpopulations within former Northern Region and CSIR-ARI flock

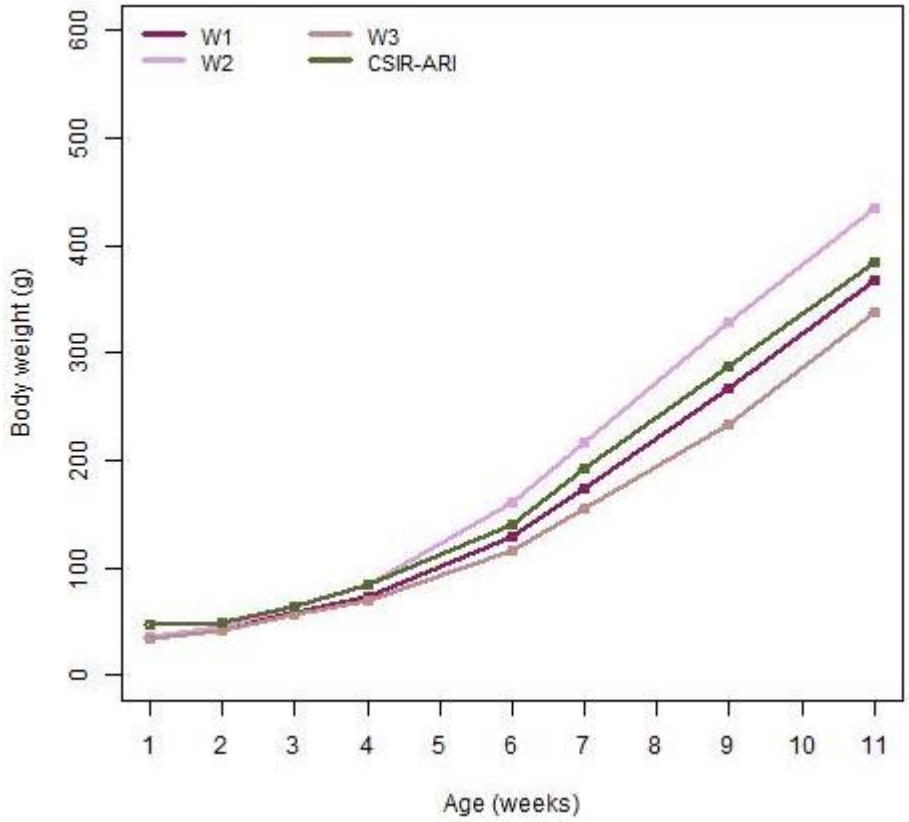


Figure 14. Growth curves for guinea fowls from subpopulations within the Upper West Region and CSIR-ARI flock

4.4.3. Growth Rates

Mean growth rates among the TPNG together with the experimental flock at CSIR-ARI are given in Table 4. Growth rates between the first and second week did not differ significantly ($p > 0.05$). Variation in growth rate was significant ($p < 0.05$) between some main populations during the period between weeks 2 to 4. Beyond the sixth week growth rates did not differ among guinea fowls from the three main populations and CSIR-ARI flock.

However the variation in growth rates within the subpopulations was significant ($p < 0.05$) beyond the second week (Table 5). Between the first and second week, birds from the subpopulations grew at similar rates. Performance of local guinea fowls from subpopulations within the main populations was different in terms of their overall growth rate.

Table 4. Comparison of growth rates (g/week) of guinea fowls from three populations of Northern Ghana and the experimental flock at CSIR-ARI

Trait		UER	FNR	UWR	CSIR-ARI
GR1^{NS}	LS Mean	$8.40^{a} \pm 1.86$	$7.41^{a} \pm 1.68$	$6.73^{a} \pm 1.44$	$1.19^{a} \pm 2.80$
	Range	4.75 - 12.05	4.10 - 10.72	3.90 - 9.56	-4.30 - 6.68
GR2***	LS Mean	$16.53^{ab} \pm 1.16$	$17.67^{a} \pm 0.99$	$13.62^{b} \pm 0.86$	$15.09^{ab} \pm 1.54$
	Range	14.24 - 18.82	15.70 - 19.63	11.93 - 15.30	12.06 - 18.12
GR3**	LS Mean	$16.15^{ab} \pm 1.55$	$14.71^{b} \pm 1.23$	$13.60^{b} \pm 1.08$	$21.18^{a} \pm 1.82$
	Range	13.10 - 19.19	12.27 - 17.14	11.48 - 15.72	17.61 - 24.75
GR4^{NS}	LS Mean	$25.07^{a} \pm 2.08$	$23.84^{a} \pm 1.59$	$25.22^{a} \pm 1.44$	$27.45^{a} \pm 2.15$
	Range	20.97 - 29.17	20.70 - 26.97	22.38 - 28.06	23.22 - 31.68
GR5^{NS}	LS Mean	$46.63^{a} \pm 3.84$	$49.55^{a} \pm 2.93$	$40.73^{a} \pm 2.76$	$49.73^{a} \pm 3.71$
	Range	39.07 - 54.19	43.77 - 55.32	35.29 - 46.18	42.43 - 57.03
GR6^{NS}	LS Mean	$42.65^{a} \pm 3.31$	$43.22^{a} \pm 2.39$	$44.68^{a} \pm 2.22$	$47.47^{a} \pm 2.86$
	Range	36.13 - 49.17	38.51 - 47.94	40.29 - 49.06	41.83 - 53.12
GR7^{NS}	LS Mean	$49.47^{a} \pm 3.32$	$47.19^{a} \pm 2.41$	$49.27^{a} \pm 2.33$	$48.03^{a} \pm 2.88$
	Range	42.91 - 56.02	42.45 - 51.94	44.67 - 53.87	42.36 -53.71
GRO***	LS Mean	$17.89^{b} \pm 1.43$	$20.92^{b} \pm 1.29$	$18.08^{b} \pm 1.11$	$31.81^{a} \pm 2.15$
	Range	15.08 - 20.69	18.38 - 23.46	15.91 - 20.26	27.60 - 36.03

GR, Growth rate; **GR1**, **GR** between weeks 1-2; **GR2**, **GR** between weeks 2-3; **GR3**, **GR** between weeks 3-4; **GR4**, **GR** between weeks 4-6; **GR5**, **GR** between weeks 6-7; **GR6, GR** between weeks 7-9, **GR7**, GR between weeks 9-11; **GRO,** overall GR between weeks 1-11; Means of populations within a row with different superscripts differ significantly at ** $p < 0.05$, ***$p < 0.001$; ^{NS} Difference of means between the populations are not statistically significant at 95% confidence level

Table 5. Growth rates (g/week) of local guinea fowls across the designated subpopulations within three populations of Northern Ghana

Subpopulation/ Pop.	GR1^{NS}	GR2**	GR3**	GR4**	GR5**	GR6**	GR7**	GRO **
E2	8.25^{a} ± 2.24	17.49^{ab} ± 1.44	16.00^{bc} ± 1.97	22.56^{c} ± 2.75	45.88^{abc} ± 5.77	42.30^{b} ± 5.52	38.40^{b} ± 5.55	14.15^{bc} ± 1.63
E3	15.60^{a} ± 11.36	27.87^{ab} ± 6.06	14.20^{abc} ± 12.15	35.30^{abc} ± 13.20	114.10^{ab} ± 23.79	45.75^{ab} ± 18.30	36.60^{ab} ± 18.41	27.08^{abc} ± 8.25
E4	8.06^{a} ± 3.53	13.49^{b} ± 1.98	16.43^{bc} ± 2.34	26.97^{bc} ± 2.64	44.35^{bc} ± 4.86	42.69^{b} ± 3.99	55.88^{ab} ± 4.02	26.27^{ae} ± 2.57
N1	4.88^{a} ± 3.28	15.39^{ab} ± 1.98	8.26^{c} ± 2.34	18.58^{c} ± 2.88	49.82^{abc} ± 6.36	41.04^{b} ± 5.08	46.56^{ab} ± 4.92	15.71^{bce} ± 2.38
N2	8.71^{a} ± 2.65	16.19^{ab} ± 1.48	13.34^{bc} ± 1.83	18.53^{c} ± 2.20	40.06^{bc} ± 4.02	40.86^{b} ± 3.23	45.35^{b} ± 3.31	20.75^{bcde} ± 1.93
N3	5.66^{a} ± 3.93	19.17^{ab} ± 2.29	16.92^{abc} ± 2.79	24.79^{bc} ± 3.41	55.60^{abc} ± 6.87	35.11^{b} ± 5.52	37.99^{b} ± 5.55	20.06^{bcde} ± 2.86
N4	10.76^{a} ± 4.51	24.77^{a} ± 2.71	29.51^{a} ± 3.37	47.75^{a} ± 3.81	72.79^{a} ± 7.17	70.83^{a} ± 6.92	71.11^{a} ± 6.96	32.41^{ad} ± 3.28
W1	6.79^{a} ± 2.29	12.79^{b} ± 1.34	13.83^{bc} ± 1.64	25.99^{bc} ± 1.99	42.47^{bc} ± 3.76	44.17^{b} ± 3.18	48.04^{ab} ± 3.54	19.02^{bce} ± 1.66
W2	7.32^{a} ± 4.77	19.15^{ab} ± 2.54	18.60^{abc} ± 3.04	37.45^{ab} ± 3.53	48.87^{abc} ± 6.60	54.35^{ab} ± 5.28	52.83^{ab} ± 5.31	32.34^{ad} ± 3.47
W3	6.57^{a} ± 2.04	13.06^{b} ± 1.20	12.18^{c} ± 1.51	20.56^{c} ± 1.99	34.47^{c} ± 4.50	41.13^{b} ± 3.46	48.92^{ab} ± 3.48	14.73^{c} ± 1.48
CSIR-ARI	1.19^{a} ± 2.81	15.09^{ab} ± 1.51	21.18^{ab} ± 1.75	27.45^{bc} ± 1.95	49.73^{abc} ± 3.55	47.47^{ab} ± 2.76	48.03^{ab} ± 2.77	31.81^{a} ± 2.04

Pop., Population; **GR**, Growth rate measured as weekly weight gain; **GR1**, **GR** between weeks 1-2; **GR2**, **GR** between weeks 2-3; **GR3**, **GR** between weeks 3-4; **GR4**, **GR** between weeks 4-6; **GR5**, **GR** between weeks 6-7; **GR6, GR** between weeks 7-9, **GR7**, GR between weeks 9-11; **GRO,** overall GR between weeks 1-11; **E2, E3, E4**, subpopulations in the Upper East Region with surviving birds; **N1, N2, N3, N4**, Subpopulations of former Northern Region; **W1,W2,W3,** Subpopulations of Upper West Region; Means of subpopulations within a column with different superscripts differ at $p < 0.05$; ^{NS} Difference between the means per subpopulation is not significant at $p > 0.05$

4.4.4. Feed Intake

The mean values for total feed intake, daily feed intake and FCR did not vary significantly ($p < 0.05$) between the main populations (Table 6). Due to the experimental design adopted by raising birds of different subpopulations together in a replicate, to avoid experimental bias originating from lower numbers of birds in some subpopulations, feed intake was not measured per subpopulation.

Table 6. Comparison of feed consumption and Feed Conversion Ratio of local guinea fowls from three populations of Northern Ghana and ARI flock

Parameter	Population			
	UER	**FNR**	**UWR**	**CSIR-ARI**
Final weight ^{NS} (g/bird)	369.05^{a}	353.52^{a}	367.39^{a}	384.09^{a}
Initial weight * (g/ bird)	37.40^{a}	34.89^{a}	35.09^{a}	46.36^{b}
Total weight gain ^{NS} (g/bird)	331.65^{a}	318.63^{a}	332.30^{a}	337.73^{a}
Average Daily Weight Gain ^{NS} (ADWG, g/bird/day)	4.74^{a}	4.55^{a}	4.75^{a}	4.82^{a}
Total feed intake ^{NS} (g)	1266.90^{a}	1315.94^{a}	1322.55^{a}	1357.67^{a}
Daily feed consumption ^{NS} (g/bird/day)	18.10^{a}	18.80^{a}	18.89^{a}	19.40^{a}
FCR^{NS}	3.82^{a}	4.13^{a}	3.98^{a}	4.02^{a}

^{NS} Variations between means within a row are not statistically significant at 95% confidence level; * Variations between means within a row are statistically significant at 95% confidence level, where means that differ bear different superscripts

4.4.5. Survivability

Highest mortalities were reported during the first week post-hatch accounting for more than 50% of total mortalities observed during the study period for each of main population and subpopulation. A summary of percentage mortalities at weekly intervals is presented in Table 7. The cumulative mortalities for TPNG by the eighth week are also given in Table 7 due to relatively high rate of mortalities recorded by local guinea fowl farmers in NG during the first 8 weeks of rearing. The survivability plots for TPNG, CSIR-ARI flock and subpopulations, CSIR-ARI flock are presented in Figures 15 and 16, respectively.

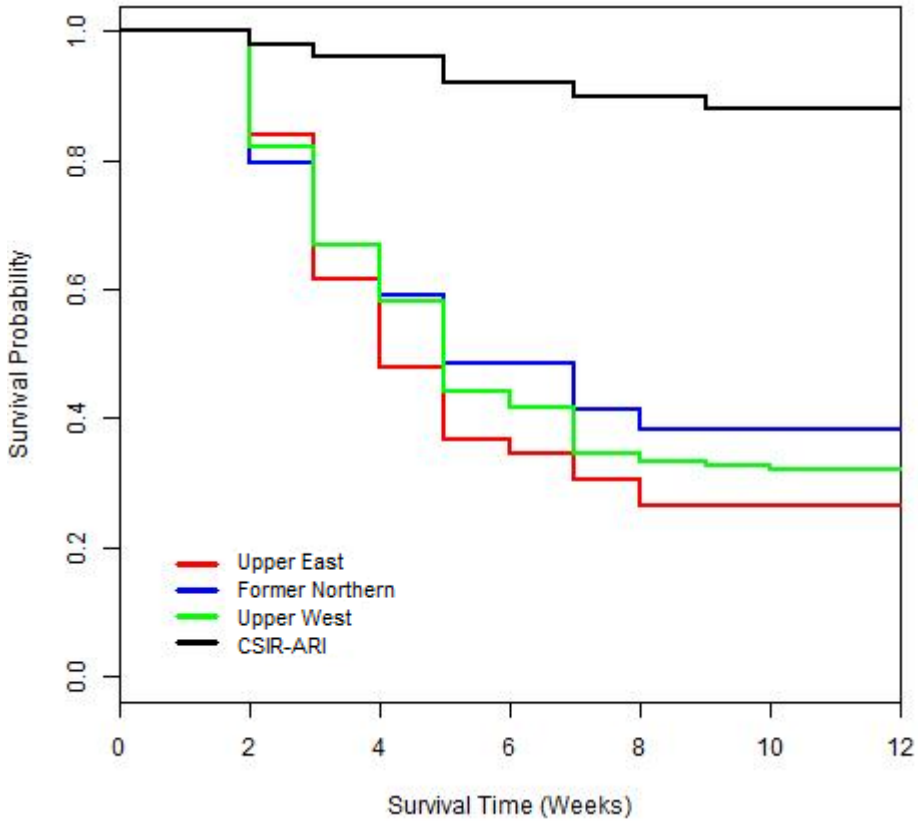


Figure 15. Survival plots for the four populations from Upper East, former Northern and Upper West Regions and CSIR-ARI flock

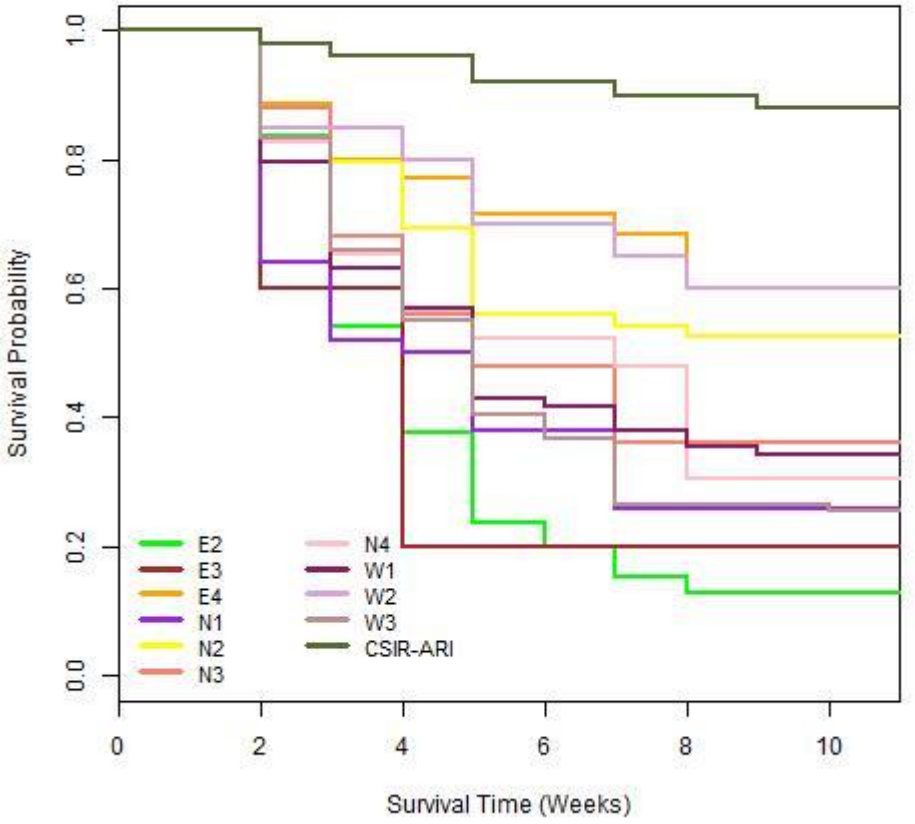


Figure 16. Survival plots for the subpopulations within the three populations of guinea fowls of NG and CSIR-ARI flock

Table 7. Percentage mortalities of guinea fowls at weekly intervals across the four populations and subpopulations

Pop. Subpop.	Initial No.	Mortalities (%)												
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Cumulative at Week 8	Week 9	Week 10	Week 11	Week 12
E1	16	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
E2	152	40.13	9.21	15.79	9.87	0.09	0.66	3.95	3.29	82.99	0.00	0.66	0.00	0.00
E3	21	76.19	9.52	0.00	9.52	0.00	0.00	0.00	0.00	95.24	0.00	0.00	0.00	0.00
E4	61	42.62	6.56	4.92	1.64	0.02	1.64	1.64	4.92	63.95	0.00	0.00	0.00	0.00
UER	250	48.00	8.00	10.80	7.20	6.00	0.80	2.80	3.20	86.80	0.00	0.40	0.00	0.00
N1	83	37.35	19.28	9.64	1.20	0.05	2.41	8.43	0.00	78.36	0.00	0.00	0.00	0.00
N2	88	29.55	7.95	5.68	6.82	9.09	0.00	1.14	3.41	63.64	0.00	1.14	0.00	0.00
N3	45	37.78	6.67	8.89	4.44	0.09	0.00	6.67	2.22	66.76	0.00	0.00	0.00	0.00
N4	27	14.81	14.81	14.81	7.41	3.70	0.00	3.70	14.81	74.07	0.00	0.00	0.00	0.00
FNR	243	32.10	12.35	8.64	4.53	0.07	0.82	4.94	3.29	66.74	0.00	0.41	0.00	0.00
W1	123	26.83	13.01	10.57	4.07	8.13	1.63	3.25	5.69	73.98	0.00	4.88	0.00	0.00
W2	36	44.44	8.33	0.00	2.78	0.03	2.78	2.78	2.78	63.92	0.00	0.00	0.00	0.00
W3	163	30.67	12.27	9.82	7.36	11.04	1.84	9.82	0.00	82.82	0.00	0.00	0.00	0.00
UWR	322	31.06	12.11	9.01	5.59	0.09	1.86	6.52	2.48	68.72	0.00	1.86	0.00	0.00
CSIR-ARI	51	1.96	1.96	0.00	0.00	1.96	0.00	1.96	0.00	7.84	1.96	0.00	0.00	0.00

Pop., Population; **Subpop.**, Subpopulation; **E2, E3, E4**, Subpopulations in the Upper East Region (UER) with surviving birds; **N1, N2, N3, N4**, Subpopulations of former Northern Region (FNR); **W1,W2,W3,** Subpopulations of Upper West Region (UWR)

4.5. Discussion

Due to the importance of carcass weight at the end of the production cycle, body weights are important quantitative traits for poultry farmers. Although guinea fowls have been improved for faster growth and higher carcass weight in France, Belgium, the USA and growth characteristics are available for some of such breeds in literature (Nahashon *et al*., 2006), there is limited literature on factors influencing body weight and early growth in guinea fowls. Most of our current understanding on factors affecting body weight during early growth stage in poultry come from the experiments involving broiler chicken breeds.

Body weight traits in broilers have been reported to be influenced by factors that have permanent or long term influence on growth such as genetic makeup of juveniles and management practices throughout the growth period (Ayorinde, 2007). However, there is also another subset of factors that influence body weight traits especially during the post-hatch growth which include maternal nutrition, breeder age, pre-incubation and incubation conditions, the effects of which subside with advancement in age in broiler chicks (Decuypere and Bruggeman, 2007).

The growth rate during the first week is usually slow due to the time taken by the chicks to increase digestive enzyme activity, feed utilization and acclimatization to life outside the egg. Therefore, body weight of chicks within the first week is largely a function of hatch weight. Several factors including maternal diet, length of pre-incubation egg storage and age of breeders have been reported to influence hatch weight and by extension body weights within the first week of age (Decuypere and Bruggeman, 2007). Nahashon *et al*. (2007) compared laying performance of guinea fowl breeders fed with diets containing varying levels of metabolizable energy and crude protein and recorded the best hatch weight in offspring from guinea hens fed with a diet including 24% and

21% crude protein between 0-8 weeks and 9-16 weeks, respectively, during the growth of breeders. This provides strong evidence for major influence of maternal diet on hatch weight and early post hatch weight in guinea fowls. Longer pre-incubation storage of eggs has also been associated with lower hatch weights (Reis *et al*., 1997; Tona *et al*., 2003; 2004) and body weight at day seven in broiler chicks (Tona *et al*., 2004).

During the current study, week one body weights (BW1) of local guinea fowls from TPNG did not vary significantly ($p > 0.05$), though birds from Upper East Region recorded the highest BW1. Keets from the experimental breeder flock at ARI had significantly heavier BW1 than keets from the TPNG. Although all the four populations were raised under identical management conditions after hatch, fed with a similar diet, the influence of the maternal diet on body weight within the first week cannot be overlooked. According to Avornyo *et al*. (2014) the practice of maintaining a separate breeder stock is non-existent in production systems prevailing in Northern Ghana. Therefore, breeder hens are left to be fend for themselves with limited supplementary feed that included maize or millet (Avornyo *et al*., 2016). The experimental flock at CSIR-ARI represents the offspring from a base population originally from the same sample locations of NG a year earlier. However, the parents were raised under best management practices recommended for raising breeders and provided with a formulated breeder diet according to (Ahiagbe *et al.*, 2016). Therefore, difference in maternal diet might be a major contributing factor for significantly higher BW1 in the CSIR-ARI population.

Although eggs laid within the last 24 hours were requested from farmers during egg collection during the current study, the exact age of eggs could be longer due to poor record keeping. Older but fertile layers are reported to lay heavier eggs reflected in higher hatch weights of their offspring compared to younger layers with the broiler lines

(Tona *et al*., 2003; 2004). Ages of the layers in the TPNG were not available due to poor record keeping by the farmers. Therefore, better maternal nutrition and shorter pre-incubation storage might be among the contributing factors for observed higher BW1 in ARI flock.

There were significant differences between the BW2, BW3 and BW4 between some regions with no significant differences between Upper East and CSIR-ARI that recorded comparable mean body weights. There have been no previous reports on body weights during post-hatch growth compared among the TPNG for comparisons with observations of the current study. Dei *et al*. (2009) recorded slightly higher body weights at week four for local guinea fowls intensively raised within cages in a study conducted to compare different brooding options.

However, the variations in body weights observed during the current study beyond the sixth week were not statistically different among the TPNG and CSIR-ARI flock. This was contrary to observations by Agbolosu *et al*. (2012a) who reported significant differences in the overall body weights beyond 8 weeks of age for the TPNG. However, Agbolosu *et al.* (2012a) did not compare body weights at specific ages. Upper East Region was the best performing in terms of weekly body weights for most weeks during the current study compared with former Northern and Upper West Regions. Agbolosu *et al*. (2012a) also reported Upper East Region as the best performing in terms of overall body weight for growth period between eight to sixteen weeks. Avornyo *et al*. (2016) observed that the proportion of farmers providing supplementary feeding is higher in Upper East Region and included millet as a major supplementary feed in that Region. Pearl millet, the variety of millet popularly grown in Upper East Region, contains a higher protein and iron percentage compared to maize (FAO, 1995). This difference in the quality and quantity of supplementary feed probably contributed to higher body

weights from week one to four in birds from Upper East Region combined with genetic factors.

Juvenile body weights observed during the current study at a given age varied from previously reported values for similar age groups in different varieties of helmeted guinea fowls in other countries. Fajemilehin (2010) reported slightly greater values for body weight at four and eight weeks in local helmeted guinea fowl varieties in Nigeria. Mohammed and Dei (2017) observed higher values for guinea keets at eight weeks raised within cages under intensive system in the former Northern Region. Dahouda *et al.* (2007) reported higher values for 10 week old local varieties in the Republic of Benin. BW1 to BW4 observed during the current study were comparable to body weights at similar ages reported by Khairunnessa *et al.* (2016) for helmeted guinea fowl varieties in Bangladesh. Body weights of pearl grey guinea fowls that have been genetically improved for higher body weight and faster growth reported by Nahashon *et al*. (2006) in USA drastically varied from the values observed during the current study. However it should be noted that performance of populations in different experiments cannot be compared due to differences in experimental conditions.

Remarkably high values for juvenile body weights of improved varieties is undoubtedly due to the genetic gain achieved over years of selection. Greater maternal nutrition ensured in standard breeder flocks coupled with good breeder stock management may be the second dominant reason for this observed difference. Avornyo *et al.* (2016) indicated that 98% of farmers in NG depend on local varieties that have not been improved by selection. The same study indicated that farmers do not maintain a separate breeder stock even within their flocks. Irrespective of age, they are raised under extensive or semi-intensive production systems (Avornyo *et al.*, 2016) where nutrition demands of breeding hens are hardly met. These observations highlight the importance

of long term selection programmes and management of breeder stock with improved nutrition in confinement in order to increase juvenile body weights in local guinea fowls to and possibly to achieve better carcass yield.

Although the variation of weekly body weights between main populations was statistically insignificant beyond the sixth week, differences between body weights between the subpopulations were significant beyond the sixth week even at 99% confidence level. This suggests that although overall the main populations did not exhibit significant variations in body weights, there was high degree of variations among the subpopulations. These variations are also vivid when growth curves are compared among the subpopulations within a given main population. As indicated from calculated contrasts between the subpopulations, the variations between subpopulations from the FNR were prominent. Former Northern Region was the largest administrative region in NG covering larger geographical coverage that probably resulted in populations with greater variation.

Faster growth has been the primary breeding objective in most past breeding programmes in poultry (Flisar *et al*., 2014). Due to the importance of post-hatch growth to the poultry farmer, factors affecting post-hatch growth have been extensively studied for broilers. Genetic factors, post-hatch diet and early access to feed have been known to influence the weekly weight gains to the end of the growth period in chicken (Noy and Sklan, 1997) while pre-incubation storage time of eggs and age of hens have been reported to influence the weight gain during early post-hatch growth (Tona *et al*., 2004).

In the current experiment, the differences among TPNG in terms of mean weekly weight gains were significant from the second week up to the fourth week, but with no further significant variations beyond the sixth week. Keets were transported to the

brooder facility within an hour post-hatch to minimize delays and stress due to treatments such as weighing and tagging at hatch, to give them access to feed within an hour post-hatch and to nullify the effects of varying fasting times on post-hatch growth. After hatch, they were fed with the same diet ensuring equal access to feeders. Representation of keets were approximately equal in all replicates per population. Therefore, observed variations in early post-hatch growth between some populations that subsided beyond the fourth week are likely due to pre-experimental factors that have transient effects such as age of breeders and pre incubation storage of eggs possibly interacting with genetic factors.

Significant contrasts in weekly weight gains between the designated subpopulations that persisted beyond the sixth week might have been influenced by some pre-experimental conditions interacting with genetic influences. As these contrasts persisted and became dominant until the end of study period, the influence of these genetic factors are likely to play a significant role on growth rate and remain to be characterized.

Indeed genetic variations within adapted guinea fowl populations in Europe and the USA have been utilized to establish genetically stable faster growing guinea fowl breeds by commercial breeding companies. Nahashon *et al.* (2006) reported remarkably high growth rates for pearl grey guinea fowls compared to growth rates observed during current study and rates reported in similar studies across Africa due to obvious genetic gain achieved by years of selection in faster growing breeds. When compared with other studies involving unimproved local varieties elsewhere local birds from the current study areas performed better than local varieties of Bangladesh for the first three weeks with a reverse trend for the remaining weeks according to reports by Khairunnesa *et al.* (2016). However growth rates observed during the current study and those reported by

other studies cannot be compared in absolute terms due to differences in experimental, pre-experimental conditions and genetic factors that influence post-hatch growth.

A pre-requisite for funding of the current study was the distribution of grower guinea fowls to beneficiary farmers at 12 weeks. Therefore no data beyond 12 weeks could be recorded.

The current study provides strong evidence for the existence of phenotypic variations in body weight and growth traits within the local guinea fowl populations of Northern Ghana. These variations provide opportunities to select phenotypically divergent lines for growth rates from local guinea fowls. However care should be taken to retain traits related to disease resistance, fertility and vigor that are of interest to smallholder guinea fowl farmers.

Reported growth rates of improved varieties raised in France, Belgium and USA are remarkably higher but exotic varieties are generally less adapted to climatic conditions prevalent in the guinea savannah ecozone. Past poultry breeding programmes that overlooked the adaptive features of local breeds to tropical climate and production systems opting for breed replacement or crossbreeding with exotic breeds have recorded little successes (FAO, 2007a). Considering the non-sustainability of breeding programmes to disseminate improved breeder stock to smallholder farmers and observations from the current study, it is recommended that future research should further explore observed variations that exist within the local populations of guinea fowls and design breeding programmes to develop fast growing strains from local populations.

The feed intake and FCR did not vary significantly between the TPNG. To avoid experimental bias from raising birds of subpopulations in separate pens due to different

number of birds per subpopulation that survived, the birds from different subpopulations were randomly allocated to replicates of main populations of NG. Hence, the feed intake was only measured among main populations and variation in FCR and its influence on observed variations within subpopulations cannot be discussed. Agbolosu *et al*. (2012a) also observed no significant differences in feed efficiency (1/FCR) between the birds from UER, FNR and UWR.

Survivability is an important economic trait in commercial poultry production. During the current study the highest mortalities were reported during the first week in all the TPNG and subpopulations. During the first week the chicks undergo a major shift in their physiology as they change from a yolk sac dependent mode of nutrition to feeding solid feed independently (Decuypere *et al*., 2001). The additional stress created during this transformation must be a major factor predisposing keets to high mortalities during the first week. While high mortalities later in life are largely functions of genotype and management of growers, high mortality in chicken during the first week is largely affected by the quality of the day old chicks (Decuypere *et al*., 2001; Kidd, 2003). Therefore, high level of mortalities that subside with age as observed during this study is likely due to low quality of day old keets hatched from the eggs collected from the TPNG. By contrast, the CSIR-ARI flock exhibited the lowest mortalities during the first week indicating improvements in quality of keets in that group.

High rate of keet mortality during the first eight weeks post-hatch has been a persistent challenge among guinea fowl farmers in NG according to previous reports by Teye and Adam (2000) and more recent reports by Avornyo *et al*. (2016). The trend is similar across tropical Africa (Bessin *et al*., 1998; Boko *et al*., 2011; Moreki and Radikara, 2013). A similar trend was observed for the groups representing the four main

populations and subpopulations of Northern Ghana during the current study with survivability increasing beyond eighth week in all groups.

In tropical Africa majority of mortalities before the eighth week have been attributed to bad weather and diseases (Dahouada *et al*., 2008; Boko *et al*., 2011). Informed by these observations, improving the microclimate of keets during the first eight weeks with provision of heat, light, water and a well-balanced diet have been proposed and have been proved to significantly reduce mortalities (Dei *et al*., 2009; Ahiagbe *et al*., 2016; Mohammed and Dei, 2017). Although best practices during brooding have been widely disseminated in NG with some farmers adopting them, guinea fowl farmers still record high keet mortalities (Avornyo *et al*., 2014). On the other hand survivability of chicks during brooding appears to be more complex and is influenced by several factors related to breeders such as breeder strain, breeder age (Peebles *et al*., 1999), breeder nutrition (Heier *et al*., 2002), factors related to egg such as egg size (Decuypere *et al*., 2001), pre-incubation storage conditions of the eggs (Tona *et al*., 2004) and incubation conditions (Lourens *et al*., 2005) beside post-hatch brooding.

In the current study all birds were fed with a formulated diet, with provision of heat, light and adhering to strict biosafety guidelines, according to best brooding practices as per the current understanding. However, survivability was still low in keets hatched from the eggs collected from the TPNG. Although all the groups were hatched at the same time and raised under the same conditions, ARI flock exhibited remarkably low levels of mortalities compared to other groups. This flock was established from parents hatched from eggs collected a year earlier from the same locations as the birds used for the comparative growth appraisal and has not been subjected to selection. Considering the common practices of obtaining eggs from their own farm or from neighbours for incubation in Northern Ghana (Avornyo *et al*., 2016) they can be assumed to be not

significantly different in descent from the birds used for present comparative growth performance trial. However, the parents of CSIR-ARI keets were raised under best practices for breeder stock management fed with a breeder diet, a health management plan and under strict biosafety measures. Unlike the breeder flock of CSIR-ARI, laying guinea hens raised by the majority of farmers are not raised intensively, and are not provided adequate feed but are left to scavenge with the rest of the flock. The resulting inadequate maternal diet and poor maternal health may be major contributing factors to high mortalities during keet stage beside other pre-incubation factors affecting the egg and the chick quality. Improved maternal diet with supplementation of Vitamin E, D and other micronutrients has been demonstrated to directly improve immunity in chicks. Supplementation of breeder diet with vitamin and mineral premixes has resulted in increased antibody production in chicks. Similarly zinc supplementation in the breeder diet has been demonstrated to improve both cellular immunity, humoral immunity and *Escherichia coli* resistance in chicks (Kidd, 2003).

Therefore, this study provides evidence for mortalities that cannot be maintained even by the best practices of post-hatch brooding. Some farmers in Northern Ghana have also reported similar observations (Avornyo *et al*., 2014). These observations highlight the complex factors that predispose local keets to high rate of mortalities. However, the remarkable improvement observed in CSIR-ARI flock in terms of survivability suggests that such mortalities can be overcome by integrating best practices of breeder stock management, pre-incubation treatment of breeding eggs and post-hatch brooding. Observed symptoms before mortalities included paralysis and nervous-like symptoms and may suggest possible vertical transfer of pathogens from parents that could not be controlled during collection and needs further investigation.

Conclusion

Variation between the three main populations of Northern Ghana is minimal in terms of juvenile body weights and early growth rates among local guinea fowls. However, there exist variations within the main populations and among the subpopulations of local varieties for traits of juvenile body weights and growth rates that should be further explored and if possible utilized for selection to ensure sustainable management of the genetic resource of indigenous guinea fowls as an alternative to breed replacement or cross breeding with exotic breeds. The high rate of keet mortality in the first eight weeks post-hatch which is common in the birds raised by farmers in NG are likely to be better managed by integrating best practices of breeder stock management, with best practices for post-hatch brooding.

CHAPTER 5

IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN EXONIC REGIONS OF INSULIN-LIKE GROWTH FACTOR 1 GENE IN LOCAL GUINEA FOWLS FROM NORTHERN GHANA

5.1. Summary

Exonic regions of Insulin-like Growth Factor (IGF1) gene (*IGF1*) in guinea fowls (*Numida meleagris*) from three populations of northern Ghana were investigated to identify Single Nucleotide Polymorphisms within them. Genomic sequences of the four coding exons of helmeted guinea fowl (*Numida meleagris*) *IGF1* were amplified using primers originally described by Bhattacharya *et al*. (2015) for homologous chicken sequences. Amplified genomic sequences from 84 local guinea fowls from Upper East Region (n = 17), former Northern Region (n = 22) and Upper West Region (n = 45) for each coding exon were purified and sequenced. Single Nucleotide Polymorphisms (SNPs) were identified by aligning generated genomic sequences with the reference genomic sequence of guinea fowl and later with MEGA ver 7.0 for genotyping. Haplotypes representing all four coding exons were constructed. Haplotype and Haplogroups of each individual was identified. Frequencies of SNP variants, genotypes at each polymorphic SNP locus, haplotypes and haplogroups were calculated by GenAlEx software ver. 6.5 (Peakall and Smouse, 2012). Haplotype diversity was determined using Arlequin software ver. 3.1 (Excoffier and Lischer, 2010).

The genomic sequences generated represent primarily the protein coding exonic regions of IGF1 gene in guinea fowl (*gIGF1*), as well as 5'and 3' untranslated regions. Primers tested may be useful for marker development and genotyping in exonic regions of g*IGF1*. Exons 1, 3, 4 and 5 that represent the first, second, third and fourth protein coding regions according to annotation provided by NCBI in g*IGF1* are homologous to exons 1, 2, 3 and 4 in chicken counterpart. Therefore, although some differences exist in arrangement of exons between the two species, protein coding sequences remain highly conserved between chicken and guinea fowls. Percentage consensus based on nucleotide identity between the homologous sequences ranged from 97% to 99%.

In total, six biallelic SNPs were identified within the exons of g*IGF1*.These include two SNPs within 5'Untranslated Region (5'UTR), one SNP within second protein coding exon and three SNPs within 3'Untranslated Region (3'UTR) of g*IGF1*, which is present on chromosome 1. The only SNP observed within sequences coding for amino acids was synonymous.

The six SNPs were distributed in seven haplotypes present within the three populations designated as H1, H2, H3, H4, H5, H6 and H7 that were different from haplotype eight (H8) observed only among birds from a domesticated French breed. Most (99%) of the genetic variation arising from distribution of SNPs among the three guinea fowl populations of Northern Ghana (TPNG) were found within the populations. Differences in distribution of haplotypes and haplogroups among the birds from the three populations were not statistically significant ($p > 0.05$).

These trends suggest no or minimal variations of primary structure of mature IGF1 protein within and between the three guinea fowl populations of Northern Ghana. Due to involvement of 5'UTR and 3'UTR in the rate of gene expression, polymorphisms observed within these genomic regions may result in different levels of IGF1 secretion by IGF1 secreting cells that may be associated with growth related traits and remains to be investigated.

5.2. Introduction

The helmeted guinea fowl (*Numida meleagris*) is an important poultry species in Ghana and Africa at large. Meat and eggs derived from guinea fowl production serve as important sources of protein, income, insurance and play other important socio-economic roles in the livelihoods of its keepers (Dei *et al*., 2014; Avornyo *et al*., 2016). Guinea fowl is the most common poultry species in Northern Ghana (NG), i.e. the Upper East Region (UER), Upper West (UWR) and Former Northern Region (FNR) (Agbolosu *et al*., 2012a).

In these regions guinea fowls are raised predominantly under extensive and semi-intensive production systems with the large majority (98%) of farmers depending on local varieties that have not been selectively bred (Avornyo *et al.*, 2016), while rearing of guinea fowls improved for faster growth is negligible (FAO, 2014). These birds represent a reservoir of rich genetic resources for breeders in search of both productive and adaptive traits. Phenotypic characterization has provided evidence for significant differences in body weight during the grower stage among the local guinea fowl varieties (Agbolosu *et al.*, 2012a).

Growth is one of the most important quantitative traits in farm animal production, breeding and genetics. In general, poultry farmers prefer faster growing birds to increase the number of production cycles per given time and to increase the body weight attained within a short time (ALBC, 2007). Like any other quantitative trait, growth is influenced by genotypes at multiple loci in a livestock genome, environment and the interactions between the genotype and the environment (Dekkers, 2012). In birds, growth in all stages including embryonic, post hatch and grower stages are regulated by a complex network of proteins within a few neuroendocrine pathways (Amills *et al*.,

2003). At the heart of growth regulation is the somatotropic axis (Zhao *et al.*, 2004). Therefore, genes coding for the proteins of the somatotropic axis are likely to represent dominant loci among the multiple loci that influence growth. Hence, they become important candidate genes to study genetics and for marker assisted selection for faster growth of poultry species (Nie *et al*., 2005).

Insulin-like Growth Factor 1 (IGF1) is a major modulator of post-hatch and juvenile growth and is secreted by several tissues in birds during all stages of growth (McMurtry, 1998). IGF1 binds to type 1 receptors and induces signal transduction ultimately resulting in increasing DNA synthesis, amino acid synthesis, protein synthesis, stimulation of cell division and differentiation (Duclos, 2005).

Due to the pivotal role that IGF1 plays in both growth hormone dependent and independent mechanisms, it is considered as one of the key candidate genes for growth in many domestic animal species (Nie *et al*., 2005; Bhattacharya *et al*., 2015). Chicken *IGF1* (*cIGF1*) has been extensively studied using different types of DNA markers, including Restriction Fragment Length Polymorphisms (RFLPs; Nagaraja *et al.*, 2000; Pandey *et al*., 2013), Single Nucleotide Polymorphisms (SNPs; Nie *et al.*, 2005; Bhattacharya *et al.*, 2015) and their association with juvenile growth established (Amills *et al.*, 2003; Bhattacharya *et al.*, 2015).

Although *cIGF1* has been extensively studied as a growth correlated candidate gene, in chicken published literature on IGF1 gene in guinea fowl (*gIGF1*) is scanty. Until recently the genomic sequences of guinea fowl were not available to facilitate studies on *gIGF1*. To the best of my knowledge, information on well characterized SNPs within functional regions of *IGF1* and/or those associated with growth in guinea fowls are not available to date.

This study, therefore examined the exonic regions of *gIGF1* to identify the SNPs in these regions, and to determine how these SNPs are distributed in haplotypes within the three main populations of guinea fowls in NG. The study also examined the diversity of haplotypes where these SNPs are distributed to generate baseline values for SNP frequencies and heterozygosity. The estimates of diversity can be used by breeders for designing sustainable marker assisted selection programmes to complement strategies for increasing genetic gain while maintaining genetic diversity.

5.3. Materials and Methods

5.3.1. PCR Amplification of target exon regions of IGF1 gene in guinea fowls

Genomic sequences of IGF1 gene in guinea fowls were not available in public genomic databases during the laboratory phase of this study for designing guinea fowl specific primers. Genomic sequences of target exons of *gIGF1* were amplified with primers based on homologous chicken sequences originally published with GenBank accession number JN593013 that had been successful in amplification of *cIGF1* according to Bhattacharya *et al.* (2015). Details of the primers used are summarised in Table 8.

Table 8. Primers used for amplification of g*IGF1*

Primer name	Homologous chicken region	Primer Sequence (5' to 3')	Length of amplicon (bp)	Annealing temperature (°C)
IGF-1E1F	Exon 1	GCTGTTTCCTGTCTACAGTG	309	57
IGF-1E1R		CTTCAAGAAATCACAAAAGCAG		
IGF-1E2F	Exon 2	GTGAAGATGCACACTGTGTC	157	55
IGF-1E2R		TGAAGTAGAAGCCTCTGTCTC		
IGF-1E3F	Exon 3	GTAAGCCTACAGGGTATGGATC	182	60
IGF-1E3R		CTTTTGTGCTTTTGGCATATCAG		
IGF-1E4F	Exon 4	GAAGTGCATTTGAAGAATACAAG	149	59
IGF-1E4R		AGTCTTCCAATGTTTAACAAATAAT		

PCR was performed in a final volume of 15 µl containing 400 µM of each dNTP, 0.4 µM of each forward and reverse primers, 20 ng DNA template, 0.75 U TaKaRa LA-*Taq* DNA Polymerase, 1 x GC buffer I and 1.5 mM magnesium chloride (Takara Bio Inc., Shiga, Japan). The PCR conditions used for the thermal cycler (Applied Biosystems, Foster City, CA, USA) were as follows: Initial denaturation at 94°C for 10 minutes, followed by 35 cycles at 95°C for 30 seconds, annealing at the given specific

annealing temperature for 30 seconds, elongation at 74°C for 60 seconds and 74°C for 5 minutes for final elongation. PCR and all subsequent laboratory analyses were performed at the Molecular Genetics Laboratory, Wildlife Research Centre, Kyoto University, Kyoto, Japan.

Aliquots of 5 µl of amplicons were resolved on 1.5% agarose gel at 100 V for 20 min by electrophoresis in TBE buffer (1M Tris base, 1M Boric acid, 0.02 M EDTA), stained with gel red and visualized under a UV transilluminator. Size of bands was determined relative to ΦX174 DNA-*Hae* III digest as the DNA size marker (Promega Corporation, Madison, USA).

5.3.2. Sequencing of *IGF1* exonic targets

Remaining 10 µl PCR product of each *gIGF1* exonic target was purified using a commercial PCR product purification kit (Roche Diagnostics, Mannheim, Germany) following the manufacturers' protocol. Purified PCR products were sequenced in both forward and reverse directions using BigDye Terminator version 3.1. Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA). Ethanol precipitated sequencing products were then resuspended in formamide, denatured and electrophoresed in an ABI PRISM 3130xl sequencer (Applied Biosystems, Foster City, CA, USA). Genomic sequences of the exonic regions of *gIGF1* were submitted to GenBank of NCBI (Appendix 2). However, release of genomic sequences generated are pending until publication of the results of this chapter in refereed journal articles.

5.3.3. Sequence alignment and SNP discovery

Consensus genomic sequences of each exonic target were generated for each local guinea fowl (n = 84) and improved exotic breed of French origin (n = 3), by aligning the forward sequence and the reverse complement of the reverse sequence using MEGA version 7 (Kumar *et al.,* 2016). The consensus genomic sequences generated from all individuals per given target were also aligned using MEGA version 7 to identify SNPs and for genotyping the birds. Genotypes for individual birds at each locus were confirmed by reconciling the genotype with the appearance of peaks in chromatograms using BioEdit version 7.0.5.3 (Hall, 1999).

Genomic sequences generated for each target exon were aligned to the guinea fowl reference genomic sequence of chromosome 1 (GenBank accession no., NC_034409) which was published by Vignal *et al.* (2017) as part of the guinea fowl whole genome sequence and the reference genomic sequence of red jungle fowl (GenBank accession no. NC_006088.4) by Nucleotide BLAST with BLASTN 2.7.1+ version of National Centre for Biotechnology Information, United States National Library of Medicine, National Institutes of Health, Maryland, USA (NCBI) (Zhang *et al.*, 2000) to determine the location of each SNP with respect to chromosome 1 and *gIGF1* of the reference genome. Each SNP was described according to Variant Call Format (VCF) based on the guidelines of NCBI, further detailed by Danecek *et al*. (2011).

5.3.4. Construction of haplotypes and haplogroups of *gIGF1*

Haplotypes arising from SNPs of all the coding exons of *gIGF1*, referred to as *gIGF1* haplotypes, were constructed by combining haplotypes for each of coding exon for each guinea fowl sampled using Arlequin software ver 3.1 (Excoffier and Lischer, 2010).

The haplogroup describe the combination of two haplotypes present within an individual bird in diploid state. Haplogroup of each bird was determined by detecting heterozygotes at each SNP site of polymorphic exons by studying the chromatograms. Heterozygosity was validated by CodonCode Aligner software package (CodonCode Corporation, Massachusetts, USA).

5.3.5. Analysis of genotype frequencies and Molecular Variance

Frequencies of the SNPs identified and genotypes at each of six SNP loci among the three guinea fowl populations from UER, FNR and UWR were calculated by formulae 1 and 2 (Table 9), respectively, using GenAlEx software ver. 6.5 (Peakall and Smouse, 2012). Haplotype and haplogroup frequencies for each of three ecotypes were also calculated using formulae 3 and 4 (Table 9), respectively, implemented in GenAlEx software ver. 6.5 (Peakall and Smouse, 2012). Haplotype diversity was determined using the Arlequin software ver. 3.1 (Excoffier and Lischer, 2010). Association of distribution of SNP variants, genotypes at SNP loci, haplotypes, haplogroups and the three main guinea fowl populations from Northern Ghana (TPNG) were established using the Pearson Chi-Square test with the help of SPSS version 24 using the actual number of genotyped individuals (IBM Corp., IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY, USA).

Analysis of Molecular variance (AMOVA) was performed to estimate the degree of variation among and between populations with 999 permutations and to estimate F_{ST} using GenAlEx ver. 6.5. F statistics were interpreted according to Hartl and Clark (1997).

Table 9. Formulae for calculating frequencies of SNP variants, genotypes, haplotypes and haplogroups of *gIGF1*

Variable	Formula	Descriptions of parameters
SNP Frequency	$\mathbf{F_{SNP}\ A = [2(n_{AA}) + (n_{AB})]/2N}$ **(1)**	F_{SNP} **A** = Frequency of SNP **A** n_{AA} = No. of **AA** homozygotes n_{AB} = No. of **AB** heterozygotes N = No. of individuals in the target population
Genotypic frequency	$\mathbf{F_{GT} = n_{AA\ (or\ AB,BB)} /N}$ **(2)**	F_{GT} = Genotypic frequency n_{AA} = No. of individuals with the given genotype AA(or AB,BB) N = No. of individuals in the target population
Haplotype frequency	$\mathbf{F_H = [2(n_{H1H1}) + (n_{H1H2})]/2N}$ **(3)**	F_{H1} = Frequency of the given haplotype **H1** n_{HIHI} = No. of **H1H1** homozygotes n_{H1H2} = No. of **H1H2** heterozygotes N = No. of individuals in the target population
Haplogroup frequency	$\mathbf{F_{H1H1} = n_{H1H1\ (or\ H1H2,\ H2H2\ etc.,)} /N}$ **(4)**	F_{H1H1} = Frequency of haplogroup **H1H1(or H1H2,H2H2,H2H3** etc.,) n_{H1H1} = No. of individuals with the given haplogroup **H1H1(or H1H2, H2H2, H2H3)** etc., N = No. of individuals in the target population

5.4. Results

5.4.1. Genomic sequences of exonic regions of *IGF1* in guinea fowl

Variants of genomic sequences obtained for each *gIGF1* exonic target by direct sequencing were submitted to the NCBI GenBank database (Appendix 2). However, availability of genomic sequences in NCBI GenBank database and accession numbers are pending until publication of results. Details of amplified genomic regions identified by aligning generated genomic sequences with chromosome 1 and *gIGF1* reference genomic sequence (GenBank accession no, NC_034409.1) are presented in Table 10. The amplicon lengths reported in chicken by Bhattacharya *et al*. (2015) and amplicon length observed in guinea fowl by gel electrophoresis are also compared in Table 10.

5.4.2. Single Nucleotide Polymorphisms in exonic regions of guinea fowl *IGF1*

A total of six SNPs were identified by direct sequencing and subsequent alignments of genomic sequences generated from 84 local guinea fowls from three populations of Northern Ghana and three guinea fowls from a domesticated meat type guinea fowl of French origin. A summary of identified SNP markers is presented in Table 11. All the SNPs were biallelic. Therefore in total 12 SNP allelic variants were identified within six different SNPs. The six polymorphic loci at base pair (bp) positions 56,018,958, 56,018,966, 56,023,560, 56,069,114, 56,069,127 and 56,069,164 on guinea fowl chromosome 1 were denoted by the acronyms IGF1SNP1T>C, IGF1SNP2T>C, IGF1SNP3T>C, IGF1SNP4T>A, IGF1SNP5G>A and IGF1SNP6G>A respectively from 5' end to 3' end of *gIGF1* for easy presentation of SNPs within this thesis.

Table 10. Locations of guinea fowl *IGF1* exonic target sequences generated and comparison with the homologous chicken sequences

Primer name	Location on guinea fowl chromosome 1 (With reference to GenBank accession no. NC_034409)	Location in the guinea fowl *IGF1* (with reference to sequence of GenBank gene ID, 110393921)	Observed amplicon size in guinea fowl	Target region/ regions in guinea fowl *IGF1*	Homologous region in chicken	Location of homologous chicken sequences on chromosome 1 (With reference to GenBank accession no. NC_006088.4)	% consensus of generated guinea fowl targets with homologous chicken sequence
IGF-1E1F IGF-1E1R	56,018,811 bp to 56,019,119 bp	Beyond 5' UTR to 259 bp	309 bp	5' UTR and coding sequence of exon 1	5'UTR, Exon 1	55,335,253 bp to 55,335,512 bp	99 - 100%
IGF-1E2F IGF-1E2R	56,023,411 bp to 56,023,567 bp	4,551 bp to 4,707 bp	157 bp	Exon 3 or second protein coding exon	Exon 2	55,339,813 bp to 55,339,969 bp	97%
IGF-1E3F IGF-1E3R	56,058,696 bp to 56,058,877bp	39,836 bp to 40,017 bp	182 bp	Exon 4 or third protein coding exon	Exon 3	55,374,107 bp to 55,374,288 bp	99%
IGF-1E4F IGF-1E4R	56,069,023 bp to 56,069,172 bp	50,163 bp to 50,307 bp	149 bp	Coding sequence of exon 5 or fourth protein coding exon, 3'UTR	Exon 4	55,383,483 bp to 55,383,632 bp	99%

Table 11. Summary of Single Nucleotide Polymorphism markers identified within *IGF1* in guinea fowl

Reference sequence (GenBank accession no.)	SNP location on chromosome 1 (bp)	SNP location in *gIGF1* (GenBank gene ID, 110393921) (bp)	Nucleotide in reference sequence	Nucleotide in alternative allele	Codon change	Amino acid change	Genomic Region	Acronym
NC_034409	56,018,958	98	T	C	ATG>ACG	none	5'UTR of exon 1	IGF1SNP1T>C
NC_034409	56,018,966	106	T	C	TGC>CGC	none	5'UTR of exon 1	IGF1SNP2T>C
NC_034409	56,023,560	4,700	T	C	TTT>TTC	none	Coding sequences of exon 3 or the second protein coding exon	IGF1SNP3T>C
NC_034409	56,069,114	50,254	T	A	TGT>AGT	none	3'UTR of exon 5	IGF1SNP4T>A
NC_034409	56,069,127	50,267	G	A	AGG>AAG	none	3'UTR of exon 5	IGF1SNP5G>A
NC_034409	56,069,164	50,304	G	A	TTG>TTA	none	3'UTR of exon5	IGF1SNP6G>A

5.4.3. Distribution of Single Nucleotide Polymorphisms within *IGF1* exons among the local guinea fowl populations

5.4.3.1. Distribution of allelic variants

Frequencies for each of the 12 allelic variants of the six SNPs per each population is summarised in Table 12. At SNPs IGF1SNP1T>C and IGF1SNP2T>C on chromosome 1, Cytosine (C) was present in the minority while Thymine (T) was present in the majority for all three populations. Adenine (A) and Guanine (G) variants at locus IGF1SNP6G>A was distributed equally to a larger extent among the three populations. Variation in the distribution of allelic variants of the SNPs among the guinea fowls from the TPNG was not statistically significant (Table 12) at any of the polymorphic loci ($p > 0.05$).

Table 12. Allelic frequencies for allelic variants at SNP sites of *IGF1* in three local guinea populations of Northern Ghana

SNP locus on chromosome 1 (bp) (GenBank accession no., NC_034409)	Description of SNP Within the thesis	SNP variant	UER (n = 17)	FNR (n = 22)	UWR (n = 45)	χ^2 (p > 0.05)
56,018,958	IGF1SNP1T>C	**C**	0.147	0.136	0.156	0.087[NS]
		T	0.853	0.864	0.844	
56,018,966	IGF1SNP2T>C	**C**	0.147	0.136	0.156	0.087 [NS]
		T	0.853	0.864	0.844	
56,023,560	IGF1SNP3T>C	**C**	0.971	0.977	0.978	0.058 [NS]
		T	0.029	0.023	0.022	
56,069,114	IGF1SNP4T>A,	**A**	0.294	0.114	0.256	4.524 [NS]
		T	0.706	0.886	0.744	
56,069,127	IGF1SNP5G>A	**A**	0.294	0.114	0.256	4.524 [NS]
		G	0.706	0.886	0.744	
56,069,164	IGF1SNP6G>A	**A**	0.471	0.500	0.500	0.094 [NS]
		G	0.529	0.500	0.500	

[NS] Variation in distribution of the two allelic variants per locus among the main populations was not statistically significant at 95% confidence level

5.4.3.2. Genotypic frequencies

Distribution of genotypes per each polymorphic locus among guinea fowls in the three populations sampled is presented in Table 13. The genotypes CC were completely absent for polymorphic loci IGF1SNP1T>C and IGF1SNP2T>C while TT genotype was absent at IGF1SNP3T>C among the local guinea fowls. Similarly no bird had AA genotype for IGF1SNP4T>A, IGF1SNP5G>A and IGF1SNP6G>A, all of which are located within 3'UTR in *gIGF1*. The variation in distribution of genotypes between the guinea fowls among the three populations was statistically significant ($p < 0.05$) only for the genotypes observed at loci 56,069,114 bp (IGF1SNP4T>A) and 56,069,127 bp (GF1SNP5G>A) on chromosome 1.

Table 13. Frequencies of genotypes arising from SNPs in *IGF1* among the three local guinea fowl populations of Northern Ghana

SNP locus on chromosome 1 (GenBank accession no., NC_034409)	Description of SNP Within the thesis	Genotype	UER (n = 17)	FNR (n = 22)	UWR (n = 45)	χ^2
56,018,958 bp	IGF1SNP1T>C	**CC**	0.000	0.000	0.000	0.105
		TT	0.706	0.727	0.689	
		CT	0.294	0.273	0.311	
56,018,966 bp	IGF1SNP2T>C	**CC**	0.000	0.000	0.000	0.105
		TT	0.706	0.727	0.689	
		CT	0.294	0.273	0.311	
56,023,560 bp	IGF1SNP3T>C	**CC**	0.941	0.955	0.956	0.059
		TT	0.000	0.000	0.000	
		CT	0.059	0.045	0.044	
56,069,114 bp	IGF1SNP4T>A	**AA**	0.000	0.000	0.000	6.393*
		TT	0.412	0.773	0.489	
		AT	0.588	0.227	0.511	
56,069,127 bp	IGF1SNP5G>A	**AA**	0.000	0.000	0.000	6.393*
		GG	0.412	0.773	0.489	
		AG	0.588	0.227	0.511	
56,069,164 bp	IGF1SNP6G>A	**AA**	0.000	0.000	0.000	3.989
		GG	0.059	0.000	0.000	
		AG	0.941	1	1	

*Variation in distribution of the genotypes per given locus among the guinea fowls in the three populations was statistically significant at 95% significance level

5.4.3.3. Haplotypes and their frequencies

Based on the SNPs present within all four coding exons, their genotypes and the frequencies a total of seven haplotypes can be predicted using Arlequin software ver. 3.1 among guinea fowls from Northern Ghana (Table 14). The eighth haplotype was observed only in the French guinea fowl samples (n = 3). Frequencies of predicted *gIGF1* haplotypes is given in Table 15. Haplotype 4 (H4) was the most common haplotype covering almost half the population in all the three study populations (Figure 17). H2 was unique to the birds of Upper West origin and was the least common haplotype in the total study population. All other haplotypes were found in at least two populations. Haplotypes H1, H3 and H5 were common to all the three populations. Variation in distribution of haplotypes among the guinea fowls from the three populations was not statistically significant ($\chi^2 = 11.731$, $p \leq 0.05$)

Table 14. Haplotypes predicted during the current study based on *IGF1* SNPs among local guinea fowls from Northern Ghana

Haplotype	Polymorphic loci and associated SNPs in *IGF1* on chromosome 1 (GenBank accession no., NC_034409)					
	56018958 bp (IGF1SNP1T>C)	**56018966 bp (IG1SNP2T>C)**	**56023560 bp (IGF1SNP3T>C)**	**56069114 bp (IGF1SNP4T>A)**	**56069127 bp (IGF1SNP5G>A)**	**56069127 bp (IGF1SNP6G>A)**
Haplotype 1 (H1)	C	C	C	T	G	G
Haplotype 2 (H2)	C	C	C	A	A	G
Haplotype3 (H3)	T	T	C	T	G	G
Haplotype4 (H4)	T	T	C	T	G	A
Haplotype5 (H5)	T	T	C	A	A	G
Haplotype 6 (H6)	T	T	T	A	A	G
Haplotype7 (H7)	T	T	T	T	G	A
Haplotype8 (H8)$^{\phi}$	T	T	T	T	G	G

A:Adenine, C:Cytosine, G:Guanine, T:Thymine, ----------:similar sequences,*similar sequences are not drawn to a scale, hence does not correspond to bp number, $^{\phi}$ Haplotype identified in domestic French variety of guinea fowl.

Table 15. Frequencies of predicted haplotypes among three local guinea fowl populations of Northern Ghana

Haplotype	UER (n = 17)	FNR (n = 22)	UWR (n = 45)
H1	0.147	0.136	0.122
H2	0.000	0.000	0.033
H3	0.088	0.25	0.122
H4	0.471	0.477	0.489
H5	0.265	0.114	0.211
H6	0.029	0.000	0.011
H7	0.000	0.023	0.011
H8	0.000	0.000	0.000

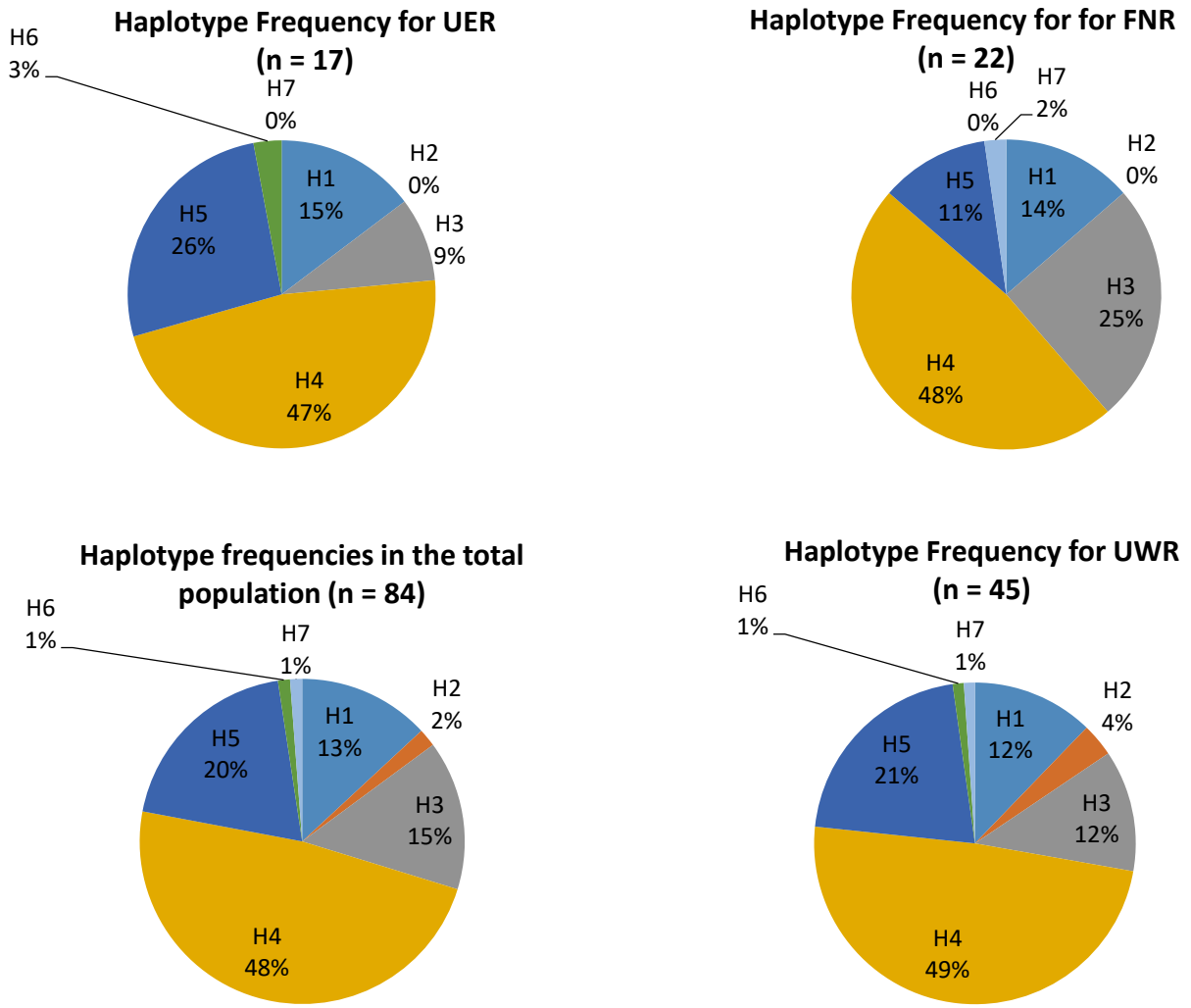


Figure 17. Comparison of percentage distribution of predicted *IGF1* haplotypes among the three main populations and the total study population

5.4.3.4. Haplogroups and their distribution

In total eight different haplogroups arising from different combinations of predicted haplotypes can be identified in the study group (Table 16).The most common haplogroup for each region differed considerably. The predominant haplogroups for Upper East Region, former Northern Region and Upper West Region were H4H5 (53%), H3H4 (46%) and H4H5 (42%), respectively. In the total population the majority of birds belonged to H4H5 followed by H3H4, H1H4, H2H4, H1H3, H4H6, H3H7 and H1H7 in the decreasing order of frequency (Figure 18). No individual local bird recorded the haplotype H8 in any of the haplogroups among the local guinea fowls from Northern Ghana. The closest among the local haplotypes to Haplotype H8 reported in French variety was H3 which only differed from H8 by a transversal synonymous mutation at 56,023,560 bp position on chromosome 1 (GenBank accession no., NC_034409). The variation of distribution of the haplogroups among the three guinea fowl populations of Northern Ghana was not statistically significant ($\chi^{2} = 18.510$, $p \geq 0.05$).

Table 16. Haplogroups identified among the study group and their haplotype combinations

Haplogroup	Polymorphic loci and associated SNPs in g*IGF1* on chromosome 1 (GenBank accession no., NC_034409)					
	56018958 bp (IGF1SNP1)	**56018966 bp (IGF1SNP2)**	**56023560 bp (IGF1SNP3)**	**56069114 bp (IGF1SNP4)**	**56069127 bp (IGF1SNP5)**	**56069127 bp (IGF1SNP6)**
H1H4	5’----------C	--------C----------C	----------T	--------G	--------G	----------3’
	5’----------T	--------T----------C	----------T	--------G	--------A	----------3’
H4H5	5’----------T	--------T----------C	----------T	--------G	--------A	----------3’
	5’----------T	--------T----------C	----------A	--------A	--------G	----------3’
H3H4	5’----------T	--------T----------C	----------T	--------G	--------G	----------3’
	5’----------T	--------T----------C	----------T	--------G	--------A	----------3’
H1H3	5’----------C	--------C----------C	----------T	--------G	--------G	----------3’
	5’----------T	--------T----------C	----------T	--------G	--------G	----------3’
H3H7	5’----------T	--------T----------C	----------T	--------G	--------G	----------3’
	5’----------T	--------T----------T	----------T	--------G	--------A	----------3’
H2H4	5’----------C	--------C----------C	----------A	--------A	--------G	----------3’
	5’----------T	--------T----------C	----------T	--------G	--------A	----------3’
H1H7	5’----------C	--------C----------C	----------T	--------G	--------G	----------3’
	5’----------T	--------T----------T	----------T	--------G	--------A	----------3’
H4H6	5’----------T	--------T ----------C	----------T	--------G	--------A	----------3’
	5’----------T	--------T----------T	----------A	--------A	--------G	----------3’

A:Adenine, C:Cytosine, G:Guanine, T:Thymine, --------:similar sequences (not drawn to a scale, hence do not correspond to bp number)

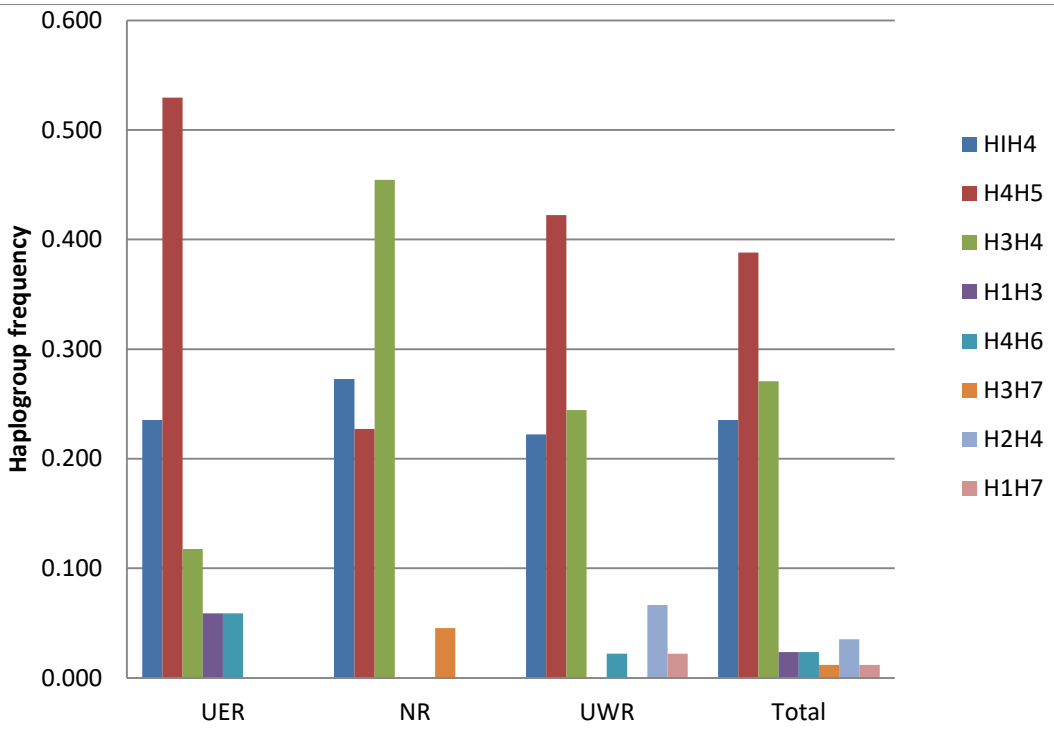


Figure 18. Distribution of haplogroups among the three guinea fowl populations sampled and the total population

5.4.4. Genetic diversity of the study population based on SNPs within *gIGF1*

The AMOVA results (Table 17) based on the distribution of allelic variants at the six different SNP sites show total variation residing within the populations to be 99% against 1% of variation among the three populations. The F_{ST} calculated based on distribution of allelic variants was 0.07 for the overall population. Pairwise comparisons of F_{ST} is presented in Table 18.

Table 17. Analysis of Molecular Variance based on the identified SNPs in three guinea fowl populations of Northern Ghana

Source of variation	d.f.	Sum of squares	Mean Square	Variance Component	% variation
Among Populations	2	1.631	0.815	0.007	1%
Between Individuals	81	39.137	0.483	0	0%
Within Populations	84	106.5	1.268	1.268	99%
Total	167	147.268		1.274	100%

Table 18. Matrix of pairwise comparisons for F_{ST} values for guinea fowls in the three populations of Northern Ghana

	UER	**FNR**	**UWR**
UER	0.000	0.129	0.367
FNR	0.025	0.000	0.147
UWR	0.000	0.014	0.000

The haplotype diversities were 0.6988 ± 0.0550, 0.6934 ± 0.0490, 0.6929 ± 0.0375 for the Upper East, former Northern and Upper West Regions, respectively. The total population had a haplotype diversity of 0.69319.

5.5. Discussion

The genomic sequences generated during the current study represent primarily the exonic regions of *gIGF1* (Figure 19). Although primers used were based on chicken genomic sequences, successful alignment of both primer and amplicon sequences with the annotated *gIGF1* sequence suggests the successful and specific amplification of intended exonic targets of *gIGF1* leading to the objectives of the study. Therefore, the primer pairs tested during this study originally proposed by Bhattacharya *et al*. (2015) for *cIGF1*, can be used for future research or downstream applications related to exonic regions in guinea fowls such as SNP genotyping, RFLP marker development and/or genotyping. Successful primer annealing that led to successful amplification also provides preliminary evidence for conservation of *IGF1* exons between guinea fowls and chicken.

Unlike in chicken where *IGF1* has been extensively studied, and for which a gene map is available (Kajimoto and Rotwein, 1989), there is no detailed literature available on the structural organization of *gIGF1* based on laboratory evidence. Messenger RNA (mRNA) variants have neither been isolated nor characterized from IGF1 secreting cells in tissues of guinea fowls. However, annotation provided by NCBI based on automated *in silico* analysis for *gIGF1* can be used to propose a tentative gene map for *gIGF1* (Chapter 2: Figure 4).

According to the annotation, *gIGF1* contains five exons unlike the *cIGF1* which contains four exons (Kajimoto and Rotwein, 1989*;* NCBI, GenBank accession no. NC_006088, Gene ID: 418090). Therefore, *gIGF1* contains an additional exon (exon 2) which is not protein coding.

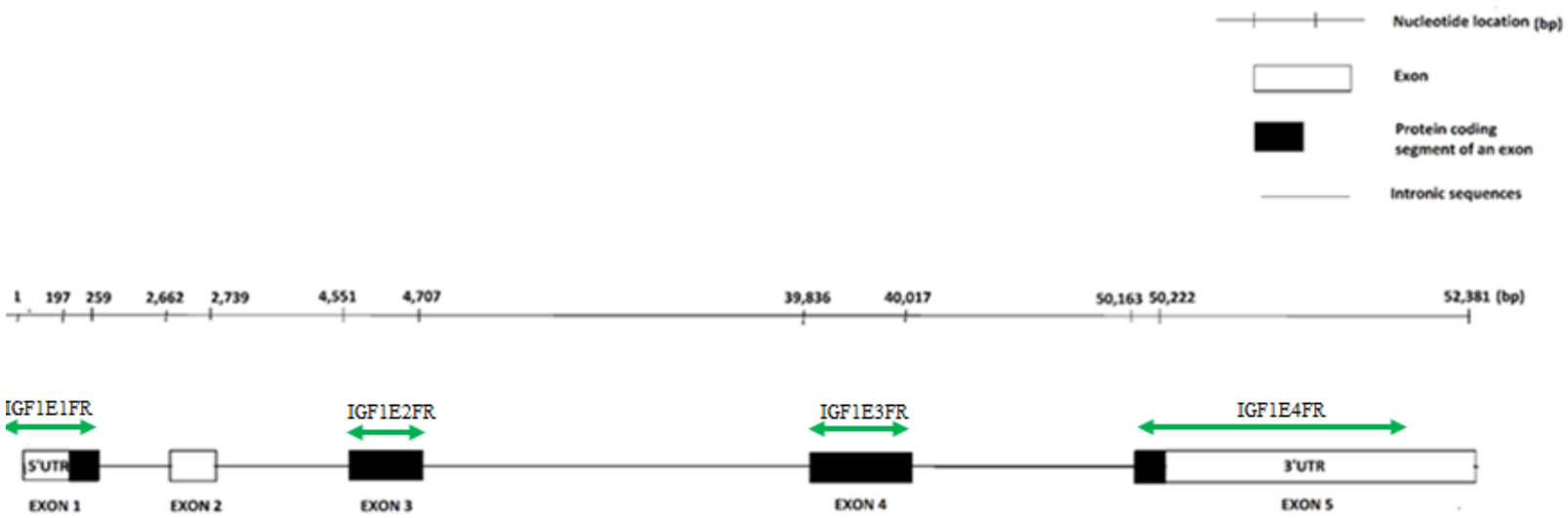
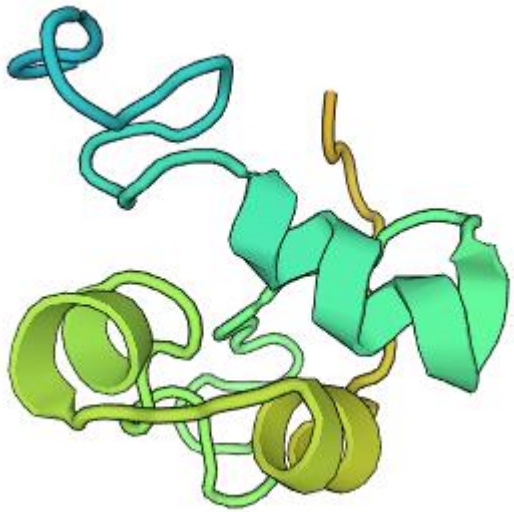


Figure 19. Schematic representation of genomic regions generated during the current study within the guinea fowl *IGF1*

BLAST results from the current study between *gIGF1* sequences generated and genomic sequence of red Jungle fowl provide direct evidence that exons 1, 3, 4 and 5 that represent actual protein coding regions in g*IGF1* are homologous to exons 1, 2, 3, and 4 in c*IGF1*.The absence of the second exon may be a contributing factor for a relatively shorter *IGF1* in chicken (48, 428bp) than in guinea fowl (52, 381 bp). Therefore, it is clear that there exist some variations in the arrangement of exonic regions in *IGF1* between chicken and guinea fowl. However, protein coding regions are highly conserved between the two species.

Genomic sequence of exon 1 of haplotype 1 of guinea fowl shared 100% identity with its homologous region of red jungle fowl indicating that this is a highly conserved region between the two species. Genomic sequences of the third exon which is the second protein coding sequence in guinea fowl shared 97% homology to the second exon of *cIGF1*. The third and fourth protein coding exons of the two species are highly conserved with 99% consensus at sequence level.

Genomic sequences of *gIGF1* generated during the current study covered the complete amino acid coding regions and 5' and 3'UTRs. The amino acid sequence coded by the genomic sequences generated predicts a 153 amino acid long chain for IGF1 protein in guinea fowl (gIGF1; Figure 20 b). Due to absence of published literature on three dimensional structures of IGF1 protein in guinea fowls, a model for three dimensional structure can be proposed for mature gIGF1 using the interactive workspace provided by SWISS MODEL (Bienert *et al.,* 2017; https://swissmodel.expasy.org/ workspace) and is given in Figure 20 which is similar to the 3D structure predicted for chicken IGF1 protein (Chapter 2: Figure 2).



(a)

```
Model_01 MEKINSLSTQLVKCCFCDFLKVKMHTVSYIHFFYLGLCLLTLTSSAAAGPETLCGAELVDALQFVCGDRGFYFSKPTGYG  80
3lri.1.A ----------------------------------------MPLSSLFVNGPRTLCGAELVDALQFVCGDRGFYFNKPTGYG  45

Model_01 SSSRRLHHKGIVDECCFQSCDLRRLEMYCAPIKPPKSARSVRAQRHTDMPKAQKEVHLKNTSRGNTGNRNYRM          153
3lri.1.A SSSRRACQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA----------------------------------          83
```

(b)

Figure 20. Proposed structure for mature IGF1 protein in guinea fowls (Source, **https://swissmodel.expasy.org/workspace**): (a), Three dimensional (3D) structure proposed for guinea fowl IGF1 protein; (b), Alignment between amino acid sequence of the mature gIGF1 protein (highlighted from blue to red from Amino terminal to Carboxy terminal) and template amino acid sequence (31ri.1.A; template used for modelling gIGF1 3D structure; NMR structure of human IGF1 in solution)

Polymorphisms found in *gIGF1* exon 1 lies within the 5' UTR. These polymorphisms can be specifically located to the 98 bp and 106 bp positions of IGF1 transcript X1 mRNA and were confirmed by performing a Nucleotide BLAST between the generated guinea fowl exon 1 sequences with its mRNA sequence (GenBank accession no., XM_021387400.1). Hence, these nucleotides do not code for amino acids. However, 5' UTR sequences have been reported to play a dominant role in post-transcriptional splicing events (Mignone *et al*., 2002; Bhattacharya *et al*., 2015). Post-transcriptional splicing has been identified as one of the key determinants of rate of gene expression (Mignone *et al*., 2002; Araujo *et al*., 2012). Therefore, the polymorphisms within *gIGF1* exon1 may yield polymorphic mRNA that may be spliced at different rates resulting in variations in the rate at which mature mRNA leave the nucleus to be translated within the ribosomes. In summary, although these substitutions do not alter the primary structure of polypeptide, they may influence the rate at which g*IGF1* is expressed by IGF1 secreting cells. Differential rate of IGF1 secretion may ultimately contribute to a quantitative difference in its biological effects such as up regulation in DNA synthesis, increasing amino acid uptake, cell division and differentiation. Indeed, high levels of available hepatic mRNA have been observed in fast growing chicken strains compared to slow growing strains (Beccavin *et al*., 2005).

The exact influence of each transcript variant arising from these on rate of splicing needs further investigation involving comparing expression profiles of animals carrying different genotypes of this polymorphism. However, possible influence of these SNPs will be dominantly manifested in homozygotes for the substitutions. Such homozygotes carrying two copies of alternative alleles were not found within the population studied. Although the sequences generated spanned the complete exon 1 including the coding region, there were no substitutions within coding segment of the exon 1. There have

been no previous reports on similar polymorphisms within 5'UTR in guinea fowls and the SNPs reported here are novel.

There have however been several reports on SNPs within 5'UTR region of *cIGF1* that appear to be more polymorphic than the protein coding sequences. Amills *et al*. (2003) reported two SNPs within 5'end of the *IGF1* in Black Penedesenca strain of chicken out of which one was present within 5'UTR. Bhattacharya *et al.* (2015) described 21 substitutions out of which 3 were located within 5'UTR of *cIGF1* using the same sets of primer pairs as in this study. Most authors who described SNPs within *cIGF1* have reported polymorphisms within 5'UTR. Therefore, the discovery of two SNPs in *gIGF1* suggests the same trend of 5'UTR being one of the most polymorphic regions within exons of avian IGF1 gene family.

Beside the 5'UTR, the promoter region located further upstream appears to be another region that exhibits polymorphism. A class of polymorphisms based on PCR-RFLP fingerprinting in *cIGF1* described by Nagaraja *et al*. (2000) in White Leghorn chicken due to A>C transversions has been reported by many authors in different chicken breeds, including coloured Indian broiler chicken (Pandey *et al*., 2013) and Wanzhai Yellow breeds (Wang *et al*., 2004). Bian *et al.* (2008) described a A>C transversion at translation initiation codon ATG at the 5'end of *cIGF1*. Ilori *et al*. (2016) identified SNPs among locally adopted chicken breeds in Nigeria.

Out of the 21 nucleotide substitutions described by Bhattacharya *et al.* (2015), seven resulted in codon changes within receptor binding domain of *cIGF1*. Four out of these were non-synonymous mutations. The trend is contrary to the current study as no amino acid altering substitutions were observed within coding sequences among the three local guinea fowl populations of Northern Ghana. The only substitution observed within the

third exon and second protein exon in *gIGF1* was a transition type and was synonymous, resulting in no changes in amino acids within the three populations studied.

Three out of the six SNPs found in the current study were located in 3'UTR. There have not been previous reports on SNPs within 3'UTR of *IGF1* in guinea fowl for comparisons. However Bian *et al.* (2008) reported a SNP at translation stop codon of c*IGF1* in chicken. Bhattacharya *et al*. (2015) reported four nucleotide substitutions within 3'UTR of c*IGF1* gene.

Nie *et al*. (2005) found 11 SNP markers within 3'UTR of *cIGF1* in a study involving Leghorn, White Recessive Rock, Taihe Silkies and Xinghua chicken breeds. Though non coding, 3'UTR regions play a major role in splicing of pre mRNA to mature mRNA that leave the nucleus for onward translation. SNPs within 3'UTR are also reported to alter the life span of mRNA molecules influencing the concentration of mRNA available for translation. This means polymorphisms within 3'UTR may alter the rate of IGF1 protein expression.

Although Adedibu *et al.* (2013) reported some indels, their positions were not specified with respect to a reference genome. There have been no other reports on SNPs in *gIGF1*. Absence of SNPs in coding sequences within *gIGF1* suggests that the primary structure is likely to be similar among the guinea fowls across the three populations with minimal variations. If the trend is similar within the larger population, this would mean that mature IGF1 protein present among local guinea fowls in Northern Ghana is very likely to be similar in primary and secondary structures, with relatively similar binding affinity to its receptor. This is different from the reports from *cIGF1* that suggest IGF1 types with varying binding affinity to its receptor among a broiler strain,

Cornish and Aseel chicken breeds studied by Bhattacharya *et al.* (2015). This may be due to genetic drift which has resulted in distant genotypes during selection of these divergent breeds unlike the ecotypes of local guinea fowls that have not been subjected to selection and are less genetically diverse, compared to highly specialized chicken breeds. Further absence of SNPs that alter amino acids between the local guinea fowls of Northern Ghana and the French guinea fowls that have been improved suggest that the two populations will express IGF1 that are structurally similar although there may be variations in IGF1 levels. This probably is due to the fact that meat-type of guinea fowls originated from the Coast of Guinea, implying that the French type of guinea fowls are relatively similar to the local guinea fowl compared to the level of divergence in chicken breeds indicating a recent ancestry of French varieties, from West African varieties also indicated by Vignal *et al*. (2017).

All SNPs identified within *gIGF1* during this study were biallelic. Although triallelic SNP variants have been reported in other growth related candidate genes, presence of only biallelic variants suggest that these loci are not hot spots for mutations. Mutations within exonic regions are considered rare phenomena (Schmid *et al*., 2005) in order for the organisms to minimize mutations that lead to alterations in biological functions of the genes. Relative abundance of SNPs within 5' and 3'UTRs compared to protein coding exons and a higher rate of transitions compared to transversions is also a trend observed throughout the avian genome (International Chicken Polymorphism Map Consortium, 2004; Schmid *et al*., 2005).

Cytosines (C) at the two polymorphic loci of 5'UTR were always found as heterozygotes, while Thymine (T) was found within heterozygotes or as homozygotes. This may be due to true absence of CC genotype in the study population due to low level of abundance of the haplotypes carrying C at these sites or inability of the CC

homozygotes to thrive to maturity. Further research with a larger population of birds is necessary to verify the reasons for these observations. Homozygotes for C at chromosomal location 56,023,560 bp included the overwhelming majority of local birds while CT was only found among three populations of local guinea fowls. It is important to note that TT at this location was only found in the exotic guinea fowls (n = 3) of French origin genotyped and the reference genomic sequence of guinea fowl from a French domestic line.

Based on AMOVA results for distribution of the six SNPs, there exist greater variation within the three populations of guinea fowls raised in Northern Ghana (99%) than between the populations. A value of 0.07 for F_{ST} for the three populations of guinea fowls sampled infers moderate level of differentiation between the populations (Hartl and Clark, 1997). Therefore, based on the distribution of the six SNPs within *gIGF1* of the TPNG, they can be best described as less structured populations that are neither largely divergent nor in complete Hardy–Weinberg equilibrium. This can mean a balance between random mating and divergence due to inbreeding, selection, genetic drift and mutations. SNPs within exonic regions are rare and are not ideal for analysis of polymorphisms such as microsatellites that can differentiate populations at a greater resolution.

The most common haplogroups among the local guinea fowls in Northern Ghana were H4H5, H3H4 and H1H4, all of which contain H4 making H4 the most common haplotype observed. Considering that mutations in exonic regions are very rare events, H4 may most probably represent the haplogroup of the early population.

Considering the key observations made on the SNP markers within protein coding exons of *gIGF1*, it is clear that variations within the polypeptide primary structure of

IGF1 isotypes are minimal among and within the guinea fowl populations raised in Upper East, Upper West, former Northern Regions of Ghana at least, within the context of the current sample size. Relatively greater polymorphism observed within 5' and 3' untranslated regions suggests possible variations in expression levels of gIGF1 that may differ among individual birds within the populations rather than between populations. Due to the significance of SNPs reported within functional regions such as the 3' and 5'UTR (Schmid *et al*., 2005), these novel SNPs reported in this chapter may be associated with growth related traits that should be investigated by extending this research. In summary, it can be proposed that IGF1 isoforms that are relatively similar in structure are found among guinea fowls in the three populations of Northern Ghana and possibly genetically predispose them to differential levels of expressions. This possibility may be extrapolated to possible variations in serum IGF1 levels and by extension differential growth rates within local guinea fowls, but this remains to be investigated.

CHAPTER 6

IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN INSULIN-LIKE GROWTH FACTOR 2 GENE IN LOCAL GUINEA FOWLS FROM NORTHERN GHANA

6.1. Summary

Insulin-like Growth Factor 2 (IGF2) is considered as an important candidate gene for embryonic and post-hatch growth in poultry. However, there are no previous reports on polymorphisms within IGF2 gene in guinea fowls (*gIGF2*). Therefore, *gIGF2* was investigated for Single Nucleotide Polymorphisms (SNPs) in three guinea fowl populations from Northern Ghana (TPNG). Target genomic regions in *gIGF2* were amplified with primers proposed by Nie *et al.* (2005) and sequenced from 84 local guinea fowls from Upper East Region (n=17), former Northern Region (n=22) and Upper West Region (n=45) of Ghana. For comparisons, samples from three exotic guinea fowls of French origin were also sequenced. SNPs were identified by aligning consensus sequences using MEGA ver. 7 and nucleotide BLAST of NCBI. Frequencies of allelic variants and genotypes were calculated using GenAlEx software ver. 6.5 (Peakall and Smouse, 2012).

Genomic sequences generated covered exon 3 and exon 4 of *gIGF2* and parts of the introns (GenBank accession no., NC_034414.1). Two novel SNPs were identified in *gIGF2* among local guinea fowls. An insertion of a Guanine (G) within a poly G motif of the intron following the third exon was identified in the majority of local guinea fowls from all TPNG. The allelic variant and the genotype present in the reference sequence and also in the three exotic guinea fowls sampled was observed in a minority of the local populations.

A substitution of G by Adenine (A) compared to the reference sequence at position 13,956,496 bp on chromosome 6 within the fourth exon which codes for most of the extension peptide of prepro IGF2 (GenBank accession no., NC_034414.1) was identified. Based on their genomic location and coding properties, these SNPs are not likely to alter the primary structure of prepro IGF2 in different genotypes.

6.2. Introduction

Growth is an important quantitative trait in animal breeding and genetics. Growth in farm animals is influenced by multiple genetic, environmental factors and their interactions (Lawrence and Fowler, 2012). The somatotropic axis plays a central role in regulation of growth and development in animals. Growth hormone secreted by the hypothalamus triggers the secretion of Insulin-like Growth Factors (IGFs) that bind to their receptors in target tissues and initiate signal transduction at cellular level that ultimately results in cell proliferation, differentiation and migration culminating in overall growth of animals (Kim, 2010).

Due to the pivotal roles played by the proteins of the somatotropic axis that include growth hormone, IGFs, their high affinity receptors and binding proteins, the genes that code for them are considered good candidate genes to study growth and development particularly during the early stages of growth in poultry. The same become ideal targets for development of molecular markers for Marker Assisted Selection (MAS) for faster growth (Nie *et al.*, 2005; Xu *et al.*, 2013).

Insulin-like Growth Factor 2 (IGF2) is one of the key components of the complex protein network of the somatotropic axis. Therefore, IGF2 gene (*IGF2*) has been considered as a major candidate gene for growth in poultry (Yan *et al.*, 2017). Several workers have studied the polymorphisms within *IGF2* in several livestock species using Single Nucleotide Polymorphisms (SNPs), due to their association with functional regions of genes and genome-wide distribution. Some associations between these SNPs and growth have also been reported.

Zwierzchowski *et al.* (2010) studied the polymorphisms in *IGF2* and their associations with some economic traits in Polish Holstein-Friesian cattle including growth rate.

Associations between SNPs, their haplotypes of *IGF2* with growth have been established in Quinchuan cattle of China (Huang *et al*., 2014). Hou *et al.* (2010) identified SNPs within porcine *IGF2* in a native Chinese pig breed and established their associations with growth.

Chicken *IGF2 (cIGF2)* is present on the fifth chromosome and is by far the most studied avian counterpart. Chicken *IGF2* has been isolated and characterized (Darling and Brickell, 1996) and the nucleotide sequence for the complete *cIGF2* is available (GenBank gene ID, 395097; GenBank accession no., NC_006092). Several workers have reported SNPs within *cIGF2* in several breeds of chicken (Amills *et al.*, 2003; Wang *et al.*, 2005; Tang *et al*., 2010).

Neither the IGF2 protein nor the gene in guinea fowl (*Numida meleagris*) (*gIGF2*) has been isolated or characterized. However Vignal *et al.* (2017) recently published the annotated genomic sequence of *gIGF2* as part of the whole genome sequence of guinea fowls. There is currently no information on the SNPs within *gIGF2* gene. This study therefore sought to identify SNPs within *gIGF2* in indigenous guinea fowl populations from Upper East Region, Upper West Region and former Northern region of Ghana.

6.3. Materials and Methods

6.3.1. Experimental Animals

Guinea fowl keets (*Numida meleagris*) hatched from the eggs collected in 34 sample locations of the Upper East Region (UER), Former Northern Region (FNR) and Upper West Region (UWR) were raised in a brooder house and fed with a formulated guinea fowl starter diet with provision of artificial heat and light up to eight weeks at the Guinea Fowl Resource Centre, CSIR-Animal Research Institute, Accra, Ghana. Birds were then raised in a deep litter house up to 11 weeks on a guinea fowl grower diet as detailed in chapters 3 and 4 of this thesis. In the 12th week 5 ml whole blood was collected aseptically from the wing vein from all surviving birds and DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA, USA). A total of 84 birds were randomly selected for SNP identification within *gIGF2*.

6.3.2. Amplification of IGF2 genomic sequences in guinea fowls by PCR and sequencing

Due to unavailability of genomic sequence of *gIGF2* in public sequence databases during laboratory phase of this study, the primers (Table 19) originally designed by Nie *et al.* (2005) to amplify homologous chicken sequences were used. An additional primer pair (IGF2E1F and IGF2E1R) was designed based on *cIGF2* genomic sequence (GenBank accession no., AH005039) using Primer 3 (Untergasser *et al.*, 2012) targeting the 5'UTR and exon 1 in *gIGF2*. The PCR reaction contained 20 ng of template DNA, 400 µM of each dNTP, 0.4 µM of each forward and reverse primers, 0.75 U TaKaRa LA-*Taq* DNA Polymerase, 1 x GC buffer I and 1.5 mM Magnesium Chloride (Takara Bio Inc., Shiga, Japan) in a final volume of 15 µl. PCR was performed with initial denaturation at 94°C for 10 minutes, followed by 35 cycles of denaturation

Table 19. Primers used for amplification of genomic targets of guinea fowl *IGF2*

Homologous region in chicken *IGF2*	Primer name	Primer Sequence (5' to 3')	Length of amplicon (bp)	Annealing temperature (°C)
5' UTR, exon 1, intron 1	IGF2E1F	CAGAGATGTGTGCTGCCAGG	339	60
	IGF2E1R	CGAAAGCAGCACTCCTCCA		
Exon 2	902F	GGTAGACCAGTGGGACGAAAT	470	60
	902R	CCTTTGGGCAACATGACATAG		
Intron 2	904F	ATCCCACTCCTATGTCATGTTGC	469	61
	904R	GGGAAGGGAGAACAACACAGTG		
3' UTR, Exon 3	903F	GGGCGAGCAGCAATGAGTAGAGG	449	68
	903R	CCGGAGCGGCGTGATGGTG		

at 95°C for 30 seconds, annealing at a specific annealing temperature (Table 19) for 30 seconds, elongation at 74°C for 60 seconds and final elongation for 10 minutes at 74°C.

Gel electrophoresis was performed to resolve 5 µl aliquots of the amplicons on 1.5% agarose gel in TBE buffer (1M Tris base, 1M Boric acid, 0.02 M EDTA) at 100V for 20 minutes. Resolved amplicons were stained with gel red and visualized under a UV transilluminator relative to ΦX174 DNA-*Hae* III digest as the DNA size marker (Promega Corporation, Madison, USA).

The PCR products of those fragments amplified successfully were purified using a commercial PCR product purification kit (Roche Diagnostics, Mannheim, Germany).The purified PCR products were sequenced using BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) in both forward and reverse directions with respective primers. Sequencing products were

resuspended in formamide and electrophoresed in an ABI PRISM 3130xl sequencer (Applied Biosystems, Foster City, CA, USA) to obtain nucleotide sequences of gene targets in both forward and reverse directions.

6.3.3. Sequence alignment and SNP discovery

Consensus genomic sequences of DNA targets were obtained by aligning forward and reverse sequences for each individual animal genotyped. In total genomic sequences of selected *IGF2* targets of 84 local guinea fowls and three exotic guinea fowls of French origin were obtained. The FASTA formatted consensus sequences from all 87 birds genotyped were aligned using MEGA 7 (Kumar *et al*., 2016) for SNP identification and genotyping. Genotypes were confirmed by reconciling the alignment and chromatograms of each bird.

Genomic sequences representing the amplified targets of *IGF2* in guinea fowls were submitted to GenBank genomic database (Appendix 3). Location of SNPs were determined by performing Nucleotide BLAST with BLASTN version 2.8.0+ of NCBI (Zhang *et al.*, 2000) with respect to reference guinea fowl sequence (GenBank accession no., NC_034414.1).

6.3.4. Data Analysis

Frequency of SNP variants and genotypes arising from polymorphisms among the three populations from UER, FNR and UWR were calculated by formulae 1 and 2 (Table 20), respectively, using GenAlEx software ver. 6.5 (Peakall and Smouse, 2012).

Table 20. Formulae for calculating frequencies of SNP variants and genotypes

Variable	Formula	Descriptions of parameters
SNP Frequency	$\mathbf{F_{SNP}\,A = [2(n_{AA}) + (n_{AB})]/2N}$ **(1)**	F_{SNP} **A** = Frequency of SNP **A** n_{AA}= No. of **AA** homozygotes n_{AB} = No. of **AB** heterozygotes N= No. of individuals in the target population
Genotypic frequency	$\mathbf{F_{GT} = n_{AA\ (or\ AB,BB)}\ /N}$ **(2)**	F_{GT} = Genotypic frequency n_{AA} = No. of individuals with the given genotype AA(or AB,BB) N = No. of individuals in the target population

6.4. Results

6.4.1. Characterization of primers and target genomic sequences

Out of the four primer pairs attempted (Table 19), the primer pairs 902 F, 902 R and 903 F, 903 R yielded specific PCR products. The remaining two primer pairs did not yield specific PCR products amidst several optimization trials. The guinea fowl DNA sequences amplified by the primers 902 F and 902 R yielded a 480 bp long genomic sequence that was mapped to exon 3 (Table 21) of the *gIGF2* and part of the adjacent intron according to computational annotation of *gIGF2* sequence (GenBank gene ID, 110400777; GenBank accession no., NC_034414.1). Genomic sequence amplified by the primers 903 F and 903 R produced a 450 bp sequence that was mapped to the protein coding segment of the exon 4 of *gIGF2* (Table 21). Locations of genomic sequences generated within *gIGF2* established by Nucleotide BLAST are represented in Figure 21. The nucleotide sequences of the genomic targets generated and submitted to GenBank database is given in Appendix 3.However, release of sequences and accession numbers are pending.

6.4.2. Single Nucleotide Polymorphisms in guinea fowl *IGF2* from Northern Ghana

Two novel Single Nucleotide Polymorphic sites including a substitution and an indel were identified within genomic targets spanning exon 3 and exon 4 of g*IGF2*, respectively, from guinea fowls sampled from the three populations of Northern Ghana. The location of each SNP with respect to reference genomic sequence of chromosome six and within *gIGF2* and other salient features of each SNP are summarised in Table 22 according to the convention used in Variant Call Format accepted by NCBI (Danecek *et al*., 2011).The indel was observed next to a Thymine (T) residue which is located at 13,955,730 bp. In the reference sequence following the T, there is a polyG

segment consisting of eight Guanine (G) residues while the alternative allele contains a nine residue long polyG segment giving rise to an insertion with respect to the reference sequence (Figure 22). The substitution 13956496G>A within exon 4 of *gIGF2* on chromosome six is a biallelic transition.

Table 21. Locations of guinea fowl IGF2 genomic targets generated and comparison with the homologous chicken sequences

Primer name	Location of generated guinea fowl sequences on chromosome 6 (With reference to GenBank accession no., NC_034414.1)	Location in the guinea fowl *IGF2* gene (with reference to sequence of GenBank gene ID, 110400777)	Observed amplicon size in guinea fowl	Target region/ regions in guinea fowl *IGF2* (According to annotation provided by NCBI for GenBank gene ID, 110400777)	GenBank Accession numbers	Homologous region in chicken	Location of Homologous region on chicken chromosome 5 (With reference to GenBank accession no. NC_006092.4)	% consensus of generated guinea fowl targets with homologous chicken sequence
902F	13,955,499 bp to 13,955,984 bp	12,566 to 13,051	486 bp	Exon 3 and a portion of intron between exons 3 and 4		Exon 3 and a portion of intron between exons 3 and 4	13,781,432 bp to 13,781,840 bp	93%
902R								
903F	13,956,345 bp to 13,956,794 bp	13,412 bp to 13,861 bp	450 bp	Exon 4 or third protein coding exon		Exon 4 or third protein coding exon	13,782,253 bp to 13,782,702 bp	96%
903R								

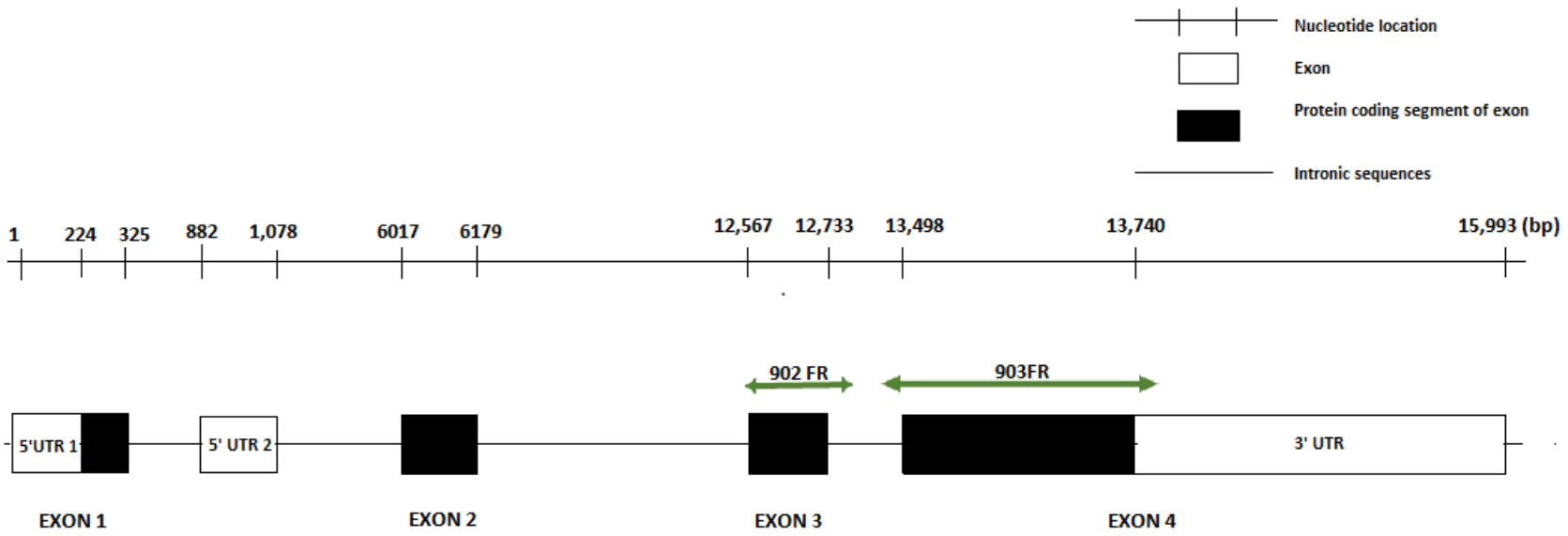


Figure 21. Locations of genomic sequences generated during the current study within *gIGF2* (*gIGF2* gene map was proposed based on annotation provided by NCBI for GenBank gene ID, 110400777)

Table 22. Summary of Single Nucleotide Polymorphic markers identified within *IGF2* gene in guinea fowl

Reference sequence (GenBank accession no.)	SNP location on chromosome 6 in guinea fowl (bp)	SNP location in *IGF2* in guinea fowl (GenBank gene ID, 110400777) (bp)	Nucleotide in reference sequence	Nucleotide/ Nucleotide sequence in alternative allele	Type of SNP variation	Genomic region
NC_034414	13,955,730	Followed by 12,797	**T**	**TG**	Indel (insertion with respect to the reference sequence)	Intron between third and fourth exon
NC_034414	13,956,496	13,563	**G**	**A**	substitution	Protein coding fourth exon

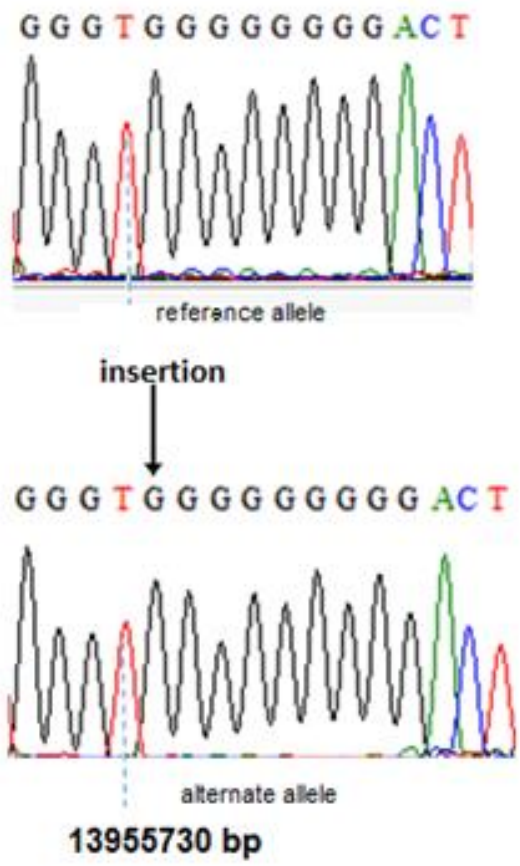


Figure 22. Appearance of chromatograms in genotypes present in the reference allele and alternative allele (with insertion). Insertion is immediately followed by the T (thymine) at 13,955,730 bp according to NCBI Nucleotide BLAST with guinea fowl reference sequence of chromosome 6 (GenBank accession no., NC_034414.1)

6.4.3. Distribution of Single Nucleotide Polymorphisms within *IGF2*

6.4.3.1. Frequency of SNP variants

Frequencies of allelic variants identified within *gIGF2* among the three guinea fowl populations from Upper East, former Northern and Upper West Region are given in Table 23. The insertion of a G in the GC rich region of the intron, observed following the 13,956,496 bp position of the guinea fowl reference sequence (GenBank accession no., NC_034414.1) was present in the majority of birds sampled in all the three populations. The distribution of allelic variants of the indel was significantly different among the three populations ($\chi^2 = 7.28$, $p < 0.05$). The 13956496G>A substitution was only present in the minority (11.9%) while the majority of individuals reported the allelic variant in the reference genome. Distribution of 13956496G>A substitution was not significantly different among guinea fowls in the three populations of Northern Ghana ($\chi^2 = 7.28$, $p > 0.05$).

Table 23. Allelic frequencies for novel SNPs identified in *IGF2* in guinea fowls from the three regions of Northern Ghana

Reference sequence	Description of SNP	SNP variant	UER (n = 17)	FNR (n = 22)	UWR (n = 45)
GenBank accession no., NC_034414.1	13956496T>TG	Null (**0**; allele reported in the reference sequence)	0.324	0.273	0.322
		Insertion (**G**; alternative allele with insertion)	0.676	0.727	0.678
GenBank accession no., NC_034414.1	13956496G>A	**A**	0.176	0.136	0.089
		G	0.824	0.864	0.911

6.4.3.2. Genotypic frequencies

The 13956496G>A substitution gave rise to three genotypes **GG, AA** and **GA** (Table 24) within the populations. Majority of birds carried GG genotype in all the three regions with no significant difference in their distribution across regions ($\chi^2 = 3.64$, $p > 0.05$). The homozygote genotype for the alternative allele (AA) was only seen in one individual from the Upper West Region.

The indel was distributed in the three genotypes **GG** (homozygote for the alternative allele or insertion reported here), **G0** (heterozygotes carrying the allele in the reference genome, **0** and the insertion, **G**), and **00** (homozygotes for allele present only in the reference sequence). The distribution was not statistically different ($p > 0.05$) but had a higher likelihood ratio of 9.296 ($p \leq 0.05$) for varied distribution. The genotype present in the reference sequence and also in the exotic guinea fowls (n = 3) of French origin was only present in the minority of the birds in all the three populations of Northern Ghana. Distribution of genotypes among the subpopulations could not be accurately interpreted due to low numbers of birds for some genotypes.

Table 24. Genotypic frequencies for SNPs in *IGF2* in local guinea fowls from the three populations of Northern Ghana

SNP	Genotype	UER (n=17)	FNR (n=22)	UWR (n=45)
Indel	**GG**	0.235	0.636	0.600
	G0	0.529	0.182	0.289
	00	0.235	0.182	0.111
G > A Substitution	**GG**	0.647	0.864	0.778
	GA	0.353	0.136	0.200
	AA	0.000	0.000	0.022

6.4. Discussion

The primers used during this study were those originally designed by Nie *et al.* (2005) to amplify *IGF2* genomic targets in chicken. The genomic regions in guinea fowls corresponding to exon 3 and exon 4 were successfully amplified by 902 F, 902R and 903F, 903R, respectively. Perfect alignment of the nucleotide sequences of these amplicons with the annotated guinea fowl reference sequence of chromosome six confirms the identity of the generated genomic sequences and the specificity of primers to avian *IGF2*. Therefore, primers 902F, 902R and 903F, 903R can be utilized for studying and genotyping exon 3 and exon 4 in guinea fowls. However, the other primers were not successful in amplifying homologous regions within *gIGF2*. This may be due to complete absence of complementary primer binding sites within the homologous genomic sequences or dissimilarities of homologous sequences. This suggests that these regions exhibit greater sequence variation between the two species compared to sequences spanning exon 3 and exon 4.

The genomic sequences amplified from primer pairs 902F, 902R were mapped to a region spanning exon 3 and beyond including part of the adjacent intron in *IGF2* in both guinea fowl (Figure 21) and chicken inferring a higher degree of homology of these sequences between the two species. However, the guinea fowl amplicon was 487 bp long compared to the homologous chicken sequence which was 410 bp long. This region covers amino acids coding exon 3 present in both Insulin-like Growth Factor 2 isoform X1 (GenBank accession no., XP_015142011.1) and Insulin-like Growth Factor 2 precursor (GenBank accession no., NP_001025513.1). A BLASTX performed with BLASTX 2.2.29+ interface of UniProt Protein database (The UniProt Consortium, 2017) revealed that this genomic

sequence is homologous to chicken sequences coding for amino acid residues 53 to 107 including part of mature protein and 15 residues of E peptide. Although guinea fowl IGF2 protein sequence has not been isolated or sequenced so far, translation of mRNA predicts a similar amino acid sequence.

Genomic sequence amplified from primers 903F and 903R represents the amino acid coding fragment of the exon 4 in both chicken (UniProt accession number**,** P33717**)** and guinea fowl. Based on the BLASTX and homology to chicken sequences, it can be predicted that this region codes for the Extension peptide (E peptide). Extension peptides are present at the C-terminus of prepro proteins and get cleaved before release.

Out of the two SNPs found within *gIGF2,* the 13956496G>A substitution could be specifically located on the Extension peptide of the predicted pro *IGF2* protein. The function of E peptides in IGF2 is not clear. However, there's evidence that they play important roles in tethering the propeptides to extracellular matrix, thereby increasing autocrine functions in growth factors in mouse models (Hede *et al.*, 2012). The substitution at 13956496 bp was a transition. Consolidated report on SNPs in chicken revealed that the most substitutions within exonic regions were transitions (Schmid *et al.,* 2005). Indeed BLASTX results revealed that this did not result in an amino acid change and hence is a synonymous mutation. Therefore, it is unlikely that these can influence the bioavailability and autocrine functions of the protein. This is an example of intrinsic precautionary attributes of genomes to protect the most valuable protein coding regions from deleterious mutations and to maximize chances that all individuals receive a functional protein for survival.

The indel observed adjacent to 13,955,730 bp of the reference sequence was located within the intronic sequence between the third and fourth exons. Deletions are more common within intronic sequences than the exonic sequences (International Chicken Polymorphism Map Consortium, 2004).

An extensive review of literature revealed no other previously reported SNPs within IGF2 gene in guinea fowl and so the SNPs reported here are novel. However, there were several other SNPs reported within IGF2 genes in chicken. Wang *et al*. (2005) reported a C>G transition within the second exon within the codon, coding for the 71st amino acid in a cross between a broiler line and Tauhe Silky chicken. Yang *et al*. (2017) also reported a synonymous mutation in Langshan chicken of China. The two SNPs reported by Amills *et al*. (2003) including C>T transition within exon 3 and G>A substitution within intron 2 were not homologous to the two SNPs identified during the current study.

Majority of local birds sampled contained the insertion following 13,955,730 bp (GenBank accession no., NC_034414.1), unlike the allelic variant present in the reference sequence and the exotic birds of French origin. The insertion adds a G residue to a short GC rich segment of the intron. Although introns were initially described as "Junk DNA", recent research is unveiling the functional elements within them and their influence on gene expression including regulation of transcription and splicing control (Zhu *et al*., 2009). Enrichment of short GC rich regions have been implicated in controlling function of the next exon and transcriptional splicing among others (Khuu *et al.,* 2007). Therefore, insertion of G within this GC rich short sequence may have some influence in RNA splicing, though minimal, and remain to be elucidated by comparing the secondary structures of mRNA variants.

CHAPTER 7

ASSOCIATIONS BETWEEN SINGLE NUCLEOTIDE POLYMORPHISMS WITHIN INSULIN-LIKE GROWTH FACTOR 1 AND INSULIN-LIKE GROWTH FACTOR 2 GENES AND EARLY GROWTH TRAITS IN LOCAL GUINEA FOWLS FROM NORTHERN GHANA

7.1. Summary

Due to the potential roles of IGF1 gene (*gIGF1*) and IGF2 gene (*gIGF2*) of guinea fowls in growth regulation, the associations between the genotypes at the eight novel SNPs identified in *gIGF1* and *gIGF2* and early growth traits were examined. The effects of various genotypes present at a given polymorphic locus were estimated using the linear model $Y_{ijkl} = \mu + R_i + S_j + GTSNP_k + e_{jkl}$ where Y_{ijkl} is the given dependent variable, μ is the overall mean, P_i is the effect of i^{th} sample Population, S_j is the effect of j^{th} Sex, $GTSNP_k$ is the effect of the k^{th} genotype at each of eight SNPs within *gIGF1* or *gIGF*2 and e_{ijkl} is the random error. Cumulative effect of the haplogroup of *gIGF1* was also estimated using the linear model $Y_{ijkl} = \mu + P_i + S_j + HPG_k + e_{ijkl}$ where Y_{ijkl} is the given dependent variable, μ is the overall mean, P_i is the effect of i^{th} sample Population, S_j is the effect of j^{th} Sex and HPG_k is the k^{th} effect of IGF1 haplogroup.

The genotypes at SNP loci IGF1SNP1T>C and IGF1SNP2T>C had significant effects on the weekly body weights measured in the second week and beyond ($p < 0.05$). The polymorphisms also influenced the growth rates between the weeks 1-2, 3-4, 6-7, 7-9 and the overall growth rate ($p < 0.05$). The SNPs IGF1SNP4T>A and IGF1SNP5G>A influenced body weight traits and the growth rates from the fourth week. However, IGF1SNP3T>C and SNPs within *gIGF2* had no effects on weekly body weights and growth rates among the TPNG.

Observed effects of genotypes at SNP loci present within the 5' and 3'UTR of *gIGF1* are likely due to the influence of these SNPs on regulation of IGF1 expression at translational level. While this study provides preliminary evidence for SNP-trait associations related to juvenile growth for the SNPs within 5' and 3'UTR of *gIGF1* in three outbred populations

of local guinea fowls from Northern Ghana, their associations with body weights and growth rates during entire growth period should be further explored in structured breeding programmes with pedigreed data to evaluate their potential use in Marker Assisted Selection.

7.2. Introduction

Helmeted guinea fowl (*Numida meleagri*s) is an indigenous poultry species of the African continent whose production is vital to livelihood of guinea fowl farmers across Africa (Moreki and Radikara, 2013). In Northern Ghana (NG), where guinea fowls are predominantly raised, the majority of poultry farmers depend on Animal Genetic Resources (AnGR) of local guinea fowls (Avornyo *et al*., 2016). Although upscaling guinea fowl production has been proposed as a socioeconomic intervention to alleviate poverty in NG (Teye and Gyawu, 2001; Dei and Karbo, 2004; Avornyo *et al*., 2016), relatively slower growth rate of the local varieties have been reported as a constraint for intensification of guinea fowl production (Agbolosu *et al*., 2012a). However remarkable improvements in growth rates have been achieved through selective breeding to develop meat-type of guinea fowls in France, Belgium and the USA (Nahashon *et al*., 2006) from domestic populations originated from the present day West Africa during the colonial era (Moreki and Radikara, 2013). Despite little success being achieved over the years, stakeholders of AnGR management in African countries have opted for cross-breeding for breed improvement of poultry species including guinea fowls primarily due to the long-time taken to achieve genetic progress through selection from indigenous breeds (FAO, 2007a).

Marker Assisted selection (MAS) has been applied to complement phenotypic selection to significantly reduce the time taken to achieve genetic progress and increase selection intensity (Stock and Reents, 2013). However its application was limited to experimental crosses of domestic animals until the wide utilization of SNPs using high density SNP platforms (SNP chips) in routine genotyping in livestock breeding schemes through

Genomic Selection (GS; Eggen, 2012). The relatively low cost of genotyping thousands of markers simultaneously with the SNP chips also provide greater prospects for application of GS in the developing world provided the genotypes of local populations are represented during the development of such SNP chips. Although, no SNP chips are available at present for helmeted guinea fowl, the whole genome sequence released by Vignal *et al*. (2017) has laid the foundation for developing high density SNP platforms for this relatively less studied poultry species. However, application of MAS and GS in selection schemes aiming at increasing growth rate will only be possible if informative SNPs that are associated with growth traits in guinea fowls are available. Deciphering associations of SNPs with traits of interest at the gene level is achieved by candidate gene approach (Goddard and Hayes, 2009).

Growth in poultry is achieved by coordinated regulation of a few neuroendocrinal pathways which include the somatotropic axis. Regulatory functions of Somatotropic axis in response to Growth Hormone is implemented by two major polypeptide growth factors, Insulin-like Growth Factor 1 (IGF1) and Insulin-like Growth Factor 2 (IGF2), belonging to insulin super family of proteins (Duclos, 2005). Therefore, the genes that code for IGF1 and IGF2 have been considered ideal candidate genes to determine associations between markers and early growth traits in poultry (Bian *et al*., 2008).

IGF1 which is also a hormone with endocrine, paracrine and autocrine functions is secreted by several tissues including liver, kidney, muscles, and skeletal tissues (McMurtry, 1998). IGF1 stimulates growth by increasing cell proliferation, migration, differentiation and reducing apoptosis (Pollak *et al*., 2004). Due to the central role that IGF1 plays in growth regulation, IGF1 gene (*IGF1*) is one of the most studied candidate genes for post-hatch

and juvenile growth in chicken (Nie *et al*., 2005; Bian *et al*., 2008). Associations between SNPs within *IGF1* have been reported by several authors in chicken including Amills *et al.* (2003), Bian *et al*. (2008) and Bhattacharya *et al*. (2015).

IGF2 is the second growth factor of the Insulin-like Growth Factor family whose growth regulatory functions are less well understood compared to IGF1. IGF2 gene (*IGF2*) has also been studied as a candidate gene for growth in chicken (Amills *et al*., 2003; Wang *et al*., 2005; Yan *et al*., 2017).

However, there have been no published reports on SNPs in *gIGF1* that have been associated with growth traits in guinea fowls. Although six SNPs were identified within the *gIGF1* including two SNPs within the 5' UTR, three SNPs within 3' UTR, one SNP within the second protein coding exon and two SNPs within *gIGF2* in the current study as described in Chapters 5 and 6, they cannot be utilized in MAS unless their associations with growth related traits are known. Therefore, *gIGF1* and *gIGF2* were studied as candidate genes for early growth to determine the statistical associations of the novel SNPs identified with body weight traits and growth rates during early growth of guinea fowls from three populations from NG. Such associations would provide preliminary information to facilitate their use in MAS aiming at improving growth rate in local guinea fowls in the future. Identification of SNPs that are associated with early growth in local varieties could also facilitate representation of local breeds in SNP chips in the future.

7.3. Materials and Methods

7.3.1. Experimental Animals

Keets were hatched from guinea fowl eggs collected from 32 sample locations (Chapter 3) across NG at Animal Research Institute of the Council for Scientific and Industrial Research (CSIR-ARI), Accra, Ghana where they were brooded according to recommended guidelines up to 8 weeks. From the eighth week they were raised in a deep litter house fed with a grower diet available *ad libitum*. Body weights at weeks 1, 2, 3, 4, 6, 7, 9 and 11 were measured while growth rates between weeks 1-2, 2-3, 3-4, 4-6, 6-7, 7-9 and entire study period were determined as weekly weight gains (Chapter 4). Eighty-four surviving birds appraised for early growth were genotyped for SNPs in *gIGF1* (Chapter 5) and *g IGF2* (Chapter 6).

7.3.2. Association between Body weight traits, growth rates and individual SNPs

The effects of genotypes at each of six SNPs in *gIGF1* and the two SNPs in *gIGF2* on the dependent variables including body weights at selected weeks (1, 2, 3, 4, 6, 7, 9, 11), weekly growth rates measured at weekly intervals of 1-2, 2-3, 3-4, 6-7, 9-11 and the overall growth rate were determined using the linear model $Y_{ijkl} = \mu + P_i + S_j + GTSNP_k + e_{ijkl}$ where, Y_{ijkl} was the dependent variable per each model, μ was the overall mean for a given dependent variable, P_i the effect of i^{th} sample Population, S_j, the effect of j^{th} Sex, $GTSNP_k$, the effect of the k^{th} genotype at a given SNP and e_{ijkl}, the random error. The linear models were implemented using the statistical package R version 0.99.489 (R core group, 2016).

7.3.3. Association between Body weight traits, growth rates and Haplogroups

Additionally the associations between the body weights measured at selected ages in weeks, growth rates at selected weekly time intervals with the haplogroups within exons of g*IGF1* were also estimated using the linear model $Y_{ijkl} = \mu + P_i + S_j + HPG_k + e_{ijkl}$ where, Y_{ijkl}, was the dependent variable, μ the overall mean, P_i, the effect of i^{th} sample Population, S_j, the effect of j^{th} Sex, HPG_k, the effect of the k^{th} haplogroup of *gIGF1* and e_{ijkl}, the random error.

7.4. Results

7.4.1. Effect of SNPs within 5' UTR region of *gIGF1*

The genotypes at the SNP loci 56,018,959 bp and 56,018,966 bp on chromosome 1 (GenBank accession no. NC_034409) present within the 5'UTR had significant effects on the body weight traits measured beyond the first week ($p < 0.05$; Table 25). At both loci the genotype CT recorded higher body weights throughout the study period. Growth rates measured as weekly weight gains between the weeks 1-2, 3-4, 6-7 and 7-9 were also influenced by the genotype significantly ($p < 0.05$). The genotype at IGF1SNP1T>C and IGF1SNP2T>C also significantly influenced the overall growth rate in local guinea fowls.

Table 25. Least Squared Means of body weights and growth rates of guinea fowls from Northern Ghana for genotypes at IGF1SNP1T>C and IGF1SNP2T>C

Trait	Genotype			
	TT		CT	
	LSM	SE	LSM	SE
BW1^{NS}	38.19^{a}	0.83	39.05^{a}	1.26
BW2**	45.88^{b}	1.16	50.39^{a}	1.75
BW3*	62.79^{b}	2.20	69.76^{a}	3.32
BW4**	79.05^{b}	3.46	92.27^{a}	5.14
BW6**	124.10^{b}	7.07	150.24^{a}	10.73
BW7**	169.07^{b}	9.45	201.89^{a}	13.73
BW9***	238.53^{b}	13.30	308.35^{a}	19.03
BW11**	340.56^{b}	17.49	417.27^{a}	25.56
GR 1**	7.70^{b}	0.81	11.34^{a}	1.22
GR 2^{NS}	16.90^{a}	1.44	19.37^{a}	2.18
GR 3**	16.14^{b}	1.62	22.48^{a}	2.41
GR 4^{NS}	22.48^{a}	2.15	28.29^{a}	3.27
GR 5*	42.30^{b}	3.10	50.92^{a}	4.51
GR 6*	41.07^{b}	2.75	54.81^{a}	3.75
GR 7^{NS}	51.11^{a}	2.96	53.05^{a}	4.33
GR O***	28.42^{b}	1.66	36.27^{a}	2.51

BWn, Body weight at week **n**, **n** =1,2,3,4,6,7,9,11; **GR**, Growth rate; **GR1**, **GR** between weeks 1-2; **GR2**, **GR** between weeks 2-3; **GR3**, **GR** between weeks 3-4; **GR4**, **GR** between weeks 4-6; **GR5**, **GR** between weeks 6-7; **GR6, GR** between weeks 7-9, **GR7**, **GR** between weeks 9-11; **GRO,** overall GR between weeks 1-11; ^{NS} effect of the genotype was not significant at 95% confidence level; * effect of the genotype was significant at 95% confidence level; ** effect of genotype was significant at 99% confidence level; *** effect of genotype was significant at 99.99% confidence level; LSM, Least Squared Means; SE, Standard Error; LSM with different superscripts were significantly different within a row at the specified confidence level

7.4.2. Effect of IGF1SNP3T>C on body weight and growth rate traits

The observed body weights and growth rates did not show significant associations ($p > 0.05$) with the two genotypes CC and CT at the SNP located at 56,023,560 bp (GenBank accession no. NC_034409) on chromosome 1 within the exon 2, denoted as IGF1SNP3T>C (Table 26).

Table 26. Least Squared Means of body weights and growth rates of guinea fowls of Northern Ghana for genotypes at IGF1SNP3T>C

Trait	Genotype			
	CC		CT	
	LS Mean	SE	LS Mean	SE
BW1^{NS}	38.58^{a}	0.74	35.76^{a}	2.94
BW2^{NS}	47.25^{a}	1.07	45.81^{a}	4.25
BW3^{NS}	64.48^{a}	2.00	70.09^{a}	7.97
BW4^{NS}	81.95^{a}	3.13	99.75^{a}	12.31
BW6^{NS}	128.67^{a}	6.44	175.11^{a}	24.41
BW7^{NS}	174.97^{a}	8.53	235.71^{a}	30.96
BW9^{NS}	254.64^{a}	12.47	335.27^{a}	44.85
BW11^{NS}	357.55^{a}	16.22	445.77^{a}	58.07
GR 1^{NS}	8.66^{a}	0.75	10.05^{a}	3.00
GR 2^{NS}	17.24^{a}	1.28	24.29^{a}	5.09
GR 3^{NS}	17.33^{a}	1.45	29.62^{a}	5.69
GR 4^{NS}	23.26^{a}	1.93	37.6^{a}	7.32
GR 5^{NS}	43.81^{a}	2.79	60.45^{a}	10.12
GR 6^{NS}	45.14^{a}	2.62	50.69^{a}	8.99
GR 7^{NS}	51.44^{a}	2.65	55.25^{a}	9.51
GR O^{NS}	30.04^{a}	1.52	41.89^{a}	6.04

BWn, Body weight at week **n**, **n** =1,2,3,4,6,7,9,11; **GR**, Growth rate; **GR1**, **GR** between weeks 1-2; **GR2**, **GR** between weeks 2-3; **GR3**, **GR** between weeks 3-4; **GR4**, **GR** between weeks 4-6; **GR5**, **GR** between weeks 6-7; **GR6, GR** between weeks 7-9, **GR7**, **GR** between weeks 9-11; **GRO,** overall GR between weeks 1-11; ^{NS} effect of the genotype was not significant at 95% confidence level; LSM, Least Squared Means; SE, Standard Error; LSM with different superscripts were significantly different within a row at the specified confidence level

7.4.3. Effect of SNPs within 3' UTR region of *gIGF1*

Both IGF1SNP4T>A and IGF1SNP5G>A located within the 3'UTR of *gIGF1* at 56,069,114 bp and 56,069,127 bp locations, respectively (GenBank accession no. NC_034409), had significant effects on the body weights from the fourth week up to 11th week (Table 27). The birds with the genotype TT at IGF1SNP4 and GG genotype at IGF1SNP5 recorded higher body weights compared to AT and AG genotypes throughout the study. The effects of genotypes observed at IGF1SNP6 could not be estimated as one of the two genotypes present at this location was only observed in one bird. These SNPs also had significant effects on weekly growth rates from the third week and the overall growth rate. Birds with the genotype TT at IGF1SNP4 and GG genotype at IGF1SNP5 grew faster than those with heterozygous genotypes (Table 27).

Table 27. Least Squared Means of body weights and growth rates of the genotypes at IGF1SNP4T>A and IGF1SNP5G>A

Trait	IGF1SNP4T>A				IGF1SNP5G>A			
	TT		AT		GG		AG	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
BW1^{NS}	38.80^{a}	0.93	37.93^{a}	1.08	38.80^{a}	0.93	37.93^{a}	1.08
BW2^{NS}	48.23^{a}	1.32	45.70^{a}	1.54	48.23^{a}	1.32	45.70^{a}	1.54
BW3^{NS}	67.20^{a}	2.47	61.42^{a}	2.88	67.20^{a}	2.47	61.42^{a}	2.88
BW4*	87.86^{a}	3.81	75.83^{b}	4.51	87.86^{a}	3.81	75.83^{b}	4.51
BW6**	144.76^{a}	7.71	111.85^{b}	9.17	144.76^{a}	7.71	111.85^{b}	9.17
BW7**	195.98^{a}	9.87	150.22^{b}	12.47	195.98^{a}	9.87	150.22^{b}	12.47
BW9***	293.33^{a}	14.01	210.55^{b}	16.69	293.33^{a}	14.01	210.55^{b}	16.69
BW11***	404.65^{a}	18.15	300.68^{b}	21.93	404.65^{a}	18.15	300.68^{b}	21.93
GR 1^{NS}	9.44^{a}	0.93	7.76^{a}	1.09	9.44^{a}	0.93	7.76^{a}	1.09
GR 2^{NS}	18.97^{a}	1.60	15.72^{a}	1.86	18.97^{a}	1.60	15.72^{a}	1.86
GR 3*	20.61^{a}	1.78	14.26^{b}	2.10	20.61^{a}	1.78	14.26^{b}	2.10
GR 4**	28.22^{a}	2.31	18.08^{b}	2.75	28.22^{a}	2.31	18.08^{b}	2.75
GR 5*	49.62^{a}	3.26	36.92^{b}	4.12	49.62^{a}	3.26	36.92^{b}	4.12
GR 6***	51.63^{a}	2.80	35.22^{b}	3.55	51.63^{a}	2.80	35.22^{b}	3.55
GR 7*	55.58^{a}	3.13	45.81^{b}	3.78	55.58^{a}	3.13	45.81^{b}	3.78
GR O***	35.10^{a}	1.76	24.51^{b}	2.06	35.10^{a}	1.76	24.51^{b}	2.06

BWn, Body weight at week **n**, **n** =1,2,3,4,6,7,9,11; **GR**, Growth rate; **GR1**, **GR** between weeks 1-2; **GR2**, **GR** between weeks 2-3; **GR3**, **GR** between weeks 3-4; **GR4**, **GR** between weeks 4-6; **GR5**, **GR** between weeks 6-7; **GR6, GR** between weeks 7-9, **GR7**, **GR** between weeks 9-11; **GRO,** overall GR between weeks 1-11;LSM, Least Square Means; SE, Standard Error; ^{NS} effect of the genotype was not significant at 95% confidence level, * effect of the genotype was significant at 95% confidence level, ** effect of genotype was significant at 99% confidence level, *** effect of genotype was significant at 99.99% confidence level, LSMs with different superscripts were significantly different at the specified confidence level

7.4.4. Effect of the *gIGF1* haplogroup on phenotypic traits

Haplogroup of *gIGF1* had a significant ($p < 0.05$) effect on body weights at weeks 4, 6, 7, 9 and 11 (Table 28) as well as weekly growth rates between weeks 3-4, 4-6 and 7-9 (Table 29). The overall growth rate for the study period was also affected by the haplogroup ($p < 0.001$). For the first, second and third weeks the haplogroups which recorded the highest body weights were H1H7, H1H4 and H4H6, respectively. For the same weeks the lowest body weight was recorded by the haplogroup H3H7. For all the weeks at which the haplogroups had significant effects on body weights, the highest body weights were recorded by the haplogroup H1H7 while the lowest body weights were recorded by H4H5. From week 1 to 3 the highest weekly growth rate was recorded by H4H6 while H3H7 recorded the lowest (Table 29). From the fourth week, the best performing haplogroups for weekly intervals 4-6, 6-7 and 9-11 were H4H6, H3H7, H3H7 and H1H7, respectively. The haplogroup H1H7 recorded the highest average weekly growth rate for the entire period while H4H5 ranked the lowest.

7.4.5. Effect of SNPs within target regions of *gIGF2*

Both SNPs located at 13,955,730bp and 13,956,496 bp positions (GenBank accession NC_034414) had no effects on weekly body weights or weekly growth rates during the study period (Table 30).

Table 28. Least Squared Means for weekly body weights (g) across the haplogroups in *gIGF1* among local guinea fowls of Northern Ghana

Trait	H1H4	H1H7	H2H4	H3H4	H3H7	H4H5	H4H6
BW1^{NS}	39.32^{a} ± 1.42	44.39^{a} ± 6.03	35.20^{a} ± 3.58	38.36^{a} ± 1.32	31.60^{a} ± 6.10	38.54^{a} ± 1.14	33.52^{a} ± 4.31
BW2^{NS}	50.87^{a} ± 2.00	50.34^{a} ± 8.51	46.88^{a} ± 5.06	46.15^{a} ± 1.86	33.94^{a} ± 8.60	45.79^{a} ± 1.61	49.48^{a} ± 6.07
BW3^{NS}	71.73^{a} ± 3.71	76.07^{a} ± 15.76	57.33^{a} ± 9.36	63.78^{a} ± 3.44	43.81^{a} ± 15.92	61.46^{a} ± 2.99	80.21^{a} ± 11.24
BW4*	94.02^{ab} ± 5.61	130.62^{a} ± 23.85	74.92^{ab} ± 14.16	82.63^{ab} ± 5.22	55.50^{b} ± 24.09	75.44^{ab} ± 4.60	106.56^{a} ± 17.01
BW6*	148.97^{ab} ± 11.11	193.15^{a} ± 47.09	130.85^{ab} ± 28.07	138.85^{ab} ± 10.55	125.52^{ab} ± 47.72	107.03^{b} ± 9.45	189.85^{a} ± 33.70
BW7*	200.43^{a} ± 14.19	251.23^{a} ± 59.89	169.19^{ab} ± 35.83	187.69^{ab} ± 13.71	206.40^{a} ± 60.70	143.25^{b} ± 13.06	240.15^{a} ± 42.87
BW9*	309.02^{a} ± 19.35	357.64^{a} ± 81.86	255.15^{ab} ± 48.91	270.94^{ab} ± 19.69	324.84^{a} ± 83.16	200.29^{b} ± 17.25	326.70^{a} ± 58.72
BW11*	412.71^{ab} ± 25.57	482.94^{a} ± 108.06	371.68^{ab} ± 79.59	390.86^{ab} ± 26.00	406.58^{ab} ± 109.83	289.09^{b} ± 22.80	444.05^{a} ± 77.58

BWn, Body weight at week **n**, **n** =1,2,3,4,6,7,9,11; Means of haplogroups within a row with different superscripts differ significantly ($p < 0.05$); ^{NS} Comparisons among the means for haplogroups were not statistically significant at 95% confidence level; * effect of the genotype was significant at 95% confidence level

Table 29. Least Squared Means for growth rates (g/week) across the haplogroups of *gIGF1* among local guinea fowls of Northern Ghana

Trait	H1H4	H1H7	H2H4	H3H4	H3H7	H4H5	H4H6
GR1^{NS}	11.55^{a} ± 1.37	5.96^{a} ± 5.84	11.67^{a} ± 3.47	7.79^{a} ± 1.27	2.34^{a} ± 5.90	7.25^{a} ± 1.11	15.96^{a} ± 4.17
GR2^{NS}	20.86^{a} ± 2.40	25.72^{a} ± 10.20	10.45^{a} ± 6.06	17.64^{a} ± 2.22	9.87^{a} ± 10.30	15.67^{a} ± 1.93	30.73^{a} ± 7.28
GR3**	22.28^{ab} ± 2.54	54.49^{a} ± 10.80	17.54^{b} ± 6.42	18.81^{b} ± 2.36	11.63^{b} ± 10.91	13.90^{b} ± 2.08	26.39^{b} ± 7.71
GR4*	27.40^{ab} ± 3.31	31.25^{a} ± 14.05	27.80^{ab} ± 8.37	28.41^{a} ± 3.15	35.19^{a} ± 14.24	15.77^{b} ± 2.82	41.50^{a} ± 10.05
GR5^{NS}	50.95^{a} ± 4.76	59.35^{a} ± 20.10	38.12^{a} ± 12.02	46.30^{a} ± 4.60	80.64^{a} ± 20.37	35.56^{a} ± 4.38	49.86^{a} ± 14.39
GR6*	55.06^{a} ± 3.95	55.08^{a} ± 16.63	45.16^{ab} ± 9.97	47.36^{ab} ± 4.10	60.21^{a} ± 16.90	33.93^{b} ± 3.82	42.89^{ab} ± 11.93
GR7^{NS}	51.79^{a} ± 4.45	62.73^{a} ±18.79	56.53^{a} ± 13.84	59.99^{a} ± 4.52	41.02^{a} ± 19.10	44.35^{a} ± 3.96	58.54^{a} ± 13.49
GRO***	37.68^{a} ± 2.57	44.40^{a} ± 10.92	31.77^{ab} ± 6.48	33.83^{ab} ± 2.38	39.41^{a} ± 11.03	23.47^{b} ± 2.07	41.79^{a} ± 7.79

GR, Growth rate; **GR1**, **GR** between weeks 1-2; **GR2**, **GR** between weeks 2-3; **GR3**, **GR** between weeks 3-4; **GR4**, **GR** between weeks 4-6; **GR5**, **GR** between weeks 6-7; **GR6, GR** between weeks 7-9, **GR7**, GR between weeks 9-11; **GRO,** overall GR between weeks 1-11; Means of haplogroups within a row with different superscripts differ significantly at *p < 0.05,**p < 0.01 ***p < 0.001; ^{NS} Difference between means between the haplogroups were not statistically significant at 95% confidence level

Table 30. Least squared Means for body weights and growth rates of various genotypes of guinea fowls at the Indel and Substitution in *gIGF2*

Trait	IGF2SNP1(13,955,730bp; GenBank accession NC_034414)			IGF1SNP2 (13,956,496 bp; GenBank accession NC_034414)		
	GG	**G0**	**00**	**GG**	**GA**	**AA**
BW1^{NS}	38.60^{a} ± 0.99	38.80^{a} ± 1.25	37.39^{a} ± 1.65	38.62^{a} ± 0.84	37.92^{a} ± 1.42	37.09^{a} ± 6.02
BW2^{NS}	47.74^{a} ± 1.44	46.37^{a} ± 1.70	46.98^{a} ± 2.37	47.54^{a} ± 1.20	46.12^{a} ± 2.04	46.02^{a} ± 8.64
BW3^{NS}	66.05^{a} ± 2.70	63.22^{a} ± 3.37	63.97^{a} ± 4.44	64.38^{a} ± 2.26	66.25^{a} ± 3.84	57.94^{a} ± 16.24
BW4^{NS}	85.12^{a} ± 4.23	80.56^{a} ± 5.36	80.81^{a} ± 6.93	82.27^{a} ± 3.59	84.90^{a} ± 6.00	79.41^{a} ± 25.39
BW6^{NS}	135.92^{a} ± 8.79	125.01^{a} ± 11.29	129.12^{a} ±13.87	126.86^{a} ± 7.47	143.32^{a} ± 11.91	126.32^{a} ± 50.41
BW7^{NS}	181.60^{a} ± 11.56	178.30^{a} ± 15.15	172.41^{a} ± 18.45	172.45^{a} ± 10.00	195.28^{a} ± 15.53	157.01^{a} ± 64.06
BW9^{NS}	262.91^{a} ± 17.36	264.93^{a} ± 21.62	246.19^{a} ± 25.52	251.72^{a} ± 14.41	281.07^{a} ± 22.56	244.35^{a} ± 92.72
BW11^{NS}	366.96^{a} ± 22.56	367.08^{a} ± 27.86	350.20^{a} ± 32.89	349.04^{a} ± 18.51	400.48^{a} ± 28.79	330.41^{a} ± 118.33
GR1^{NS}	9.14^{a} ± 1.01	7.57^{a} ± 1.26	9.59^{a} ± 1.66	8.91^{a} ± 0.85	8.19^{a} ± 1.44	8.93^{a} ± 6.11
GR2^{NS}	18.31^{a} ± 1.74	16.85^{a} ± 2.18	16.98^{a} ± 2.86	16.85^{a} ± 1.44	20.13^{a} ± 2.45	11.92^{a} ± 10.38
GR3^{NS}	18.98^{a} ± 1.98	17.09^{a} ± 2.52	16.84^{a} ± 3.26	17.72^{a} ± 1.69	18.66^{a} ± 2.82	21.31^{a} ± 11.93
GR4^{NS}	25.03^{a} ± 2.64	22.45^{a} ± 3.40	24.11^{a} ± 4.17	22.16^{a} ± 2.22	29.17^{a} ± 3.54	23.38 ^{a} ± 14.99
GR5^{NS}	43.77^{a} ± 3.70	50.44^{a} ± 4.84	39.99^{a} ± 5.90	43.42^{a} ± 3.26	48.92^{a} ± 5.06	31.97^{a} ± 20.86
GR6^{NS}	48.03^{a} ± 3.45	47.68^{a} ± 4.33	37.71^{a} ± 5.11	45.11^{a} ± 3.03	46.53^{a} ± 4.59	45.51^{a} ± 18.41
GR7^{NS}	51.91^{a} ± 3.64	51.08^{a} ± 4.50	52.00^{a} ± 5.31	48.67^{a} ± 2.94	59.70^{a} ± 4.57	43.02^{a} ± 18.79
GRO^{NS}	31.44^{a} ± 2.09	29.39^{a} ± 2.61	30.70^{a} ± 3.44	28.96^{a} ± 1.70	35.83^{a} ± 2.90	29.15^{a} ± 12.26

BWn, Body weight at week **n**, **n** =1,2,3,4,6,7,9,11; **GR**, Growth rate; **GR1**, **GR** between weeks 1-2; **GR2**, **GR** between weeks 2-3; **GR3**, **GR** between weeks 3-4; **GR4**, **GR** between weeks 4-6; **GR5**, **GR** between weeks 6-7; **GR6, GR** between weeks 7-9, **GR7**, **GR7**, GR between weeks 9-11; **GRO,** overall GR between weeks 1-11; Means of genotypes within a row for a SNP with different superscripts differ significantly ($p < 0.05$); ^{NS} Comparisons among the means of genotypes per SNP were not statistically significant at 95% confidence level

7.5. Discussion

Regulation of gene expression is a complex function achieved through different stages from chromatin modification to transcription and translation. Relative contribution of any and/or each of the stages to overall gene expression differs from the type of the gene and the physiological or growth stage of an organism (Pesole *et al*., 2001). Due to the relatively short time taken for cell division, regulation of protein expression at translation level has been reported to be of particular importance in proteins involved in growth regulation, cell division and differentiation (Gebauer and Hentze, 2004). IGF1 is a major modulator of growth, cell division, differentiation and migration. Gene regulation at translation level is influenced by 5'UTR, 3'UTR and upstream Open Reading Frames (uORFs) (Pesole *et al*., 2001).

The SNPs IGF1SNP1T>C and IGF1SNP2T>C located at 56,018,959 bp and 56,018,966 bp within the 5'UTR of *gIGF1* had significant effects on body weights from the second week while, they also affected the weekly growth rates in an irregular but rhythmic pattern. Although, 5'UTRs do not code for amino acids, these upstream non-coding regions are known to influence the rate at which mRNA transcripts are translated to proteins (Bhattacharya *et al*., 2015). They play important roles in recruitment of ribosomes and initiation of translation (Gebauer and Hentze, 2004). Proteins involved in cell cycle regulation are reported to have longer and more structured 5'UTRs (Pesole *et al*., 2002).Therefore, the alternative alleles at SNP loci within the 5'UTR probably increase the rate of translation of mRNA to IGF1 protein, thereby causing the observed effects in guinea fowls. These non-coding segments of mRNA transcripts include multiple sequences that have been demonstrated to influence rate of translation including 7-methyl guanosine cap

(GCAP), secondary structures, upstream AUG codon and Ribosome Binding Protein (RBP) binding sites. However, mRNA molecules of *gIGF1* have neither been isolated nor characterized, limiting my ability to suggest a specific molecular mechanism for the observed associations of SNPs within the 5'UTR of *gIGF1*. Based on the associations observed, it can be hypothesised that these SNPs influence growth by influencing rate of translation of IGF1 protein by influencing rate of translation at one or more functional elements within the 5'UTR mentioned above.

The SNPs IGF1SNP4T>A and IGF1SNP5G>A located 13 bp apart within the 3'UTR region had significant and similar statistical effects on body weights and growth rates beyond the fourth week. Considering the complex mechanisms through which polymorphisms within 3'UTR bring about differential rates of translation, the exact contribution of each SNP may be different. Due to the adenine (A) at IGF1SNP4T>A which is in Linkage Disequilibrium (LD) with an A at IGF1SNP5G>A, each of these SNPs appears to have similar statistical effects. However, the exact functional influence may be restricted to only one of the two SNPs or to both SNPs having an integrated effect on the traits. This observation confirms that haplotype assignment of *gIGF1* described in Chapter 5 was accurate. SNP variants that contained A at IGF1SNP4 and IGF1SNP5 in LD are present in the haplotypes H2, H5 and H6.

After transcription within the nucleus mRNA enters the endoplasmic reticulum for translation. The 3'UTRs regulate translation by influencing efficiency of translation, rate of mRNA turnover (Brown and Sachs, 1998) or subcellular localization (Jansen, 2001) particularly in proteins involved in early growth (Mignone *et al*., 2002). Repression of some mRNAs occurs by micro RNA (miRNA) interacting with target regions with 3'UTR

by RNA silencing. The 3'UTRs also contain AU rich regions (AREs) that serve as target sites for deadenylation and hence degradation of mRNA molecules (Decker and Parker, 1995). IGF1 gene has been reported to have five AUUUA motifs that are conserved across several metazoan species (Oberbauer, 2013) although, there are other classes of AREs that do not contain the AUUUA pentanucleotide that have been less characterized (Mignone *et al*., 2002). 3'UTRs also contain endonuclease specific binding sites which can be sequestered by competing RNA binding proteins (Szostak and Gebauer, 2012). Therefore, polymorphisms in target AREs and target sites for endonucleases within 3'UTR of *IGF1* can alter abundance of mRNA and IGF1 levels.

The alternative alleles A at the two SNP sites within 3'UTR will give rise to a mRNA that contains addition Uracil (U) resides, but they were not found within a putative AUUUA motif. The specific functional homologues for translational regulatory elements such as different classes of AREs, binding sites for endonucleases or sequestering proteins have not been identified in mRNA variants in guinea fowls, limiting my capacity to specifically predict the molecular mechanisms for the observed influence of alternative alleles in SNPs on bodyweight traits. Therefore, elucidation of secondary structures of mRNA molecules and identification of regulatory motifs within 5'UTR and 3'UTR are recommended for future research.

Beside the statistically significant effects of these SNPs within the 3'UTR on body weights and growth rates, the degree of significance on different traits provide important evidence on apparent functional roles for polymorphisms within the 3'UTR of *gIGF1*. Significant level of statistical effects arising from SNPs on body weights was increasing with advancing age while the same varied in a rhythmic pattern though somewhat irregular.

Increasing effect of polymorphisms on bodyweights are probably due to cumulative effect of higher serum levels of IGF1 protein over the study period. Differential serum IGF1 levels hypothesised to be arising from polymorphisms within the 3'UTR would be all cleared from sera at the same rate due to a similar tertiary structure predicted by absence of SNPs within amino acid coding nucleotides for gIGF1. Therefore, the time gap between replacement of IGF1 from transcription and translation and IGF1 clearance may be a contributing factor for intermittent intervals with no influence of SNPs on growth rates. However, direct experimental evidence from serum IGF1 levels measured at weekly intervals is necessary to confirm these claims. Nevertheless, these patterns together with observed statistical effects of the SNPs on body weight and growth suggest some functional roles that are yet to be characterized for these polymorphisms in the regulation of IGF1 expression at translational levels. Therefore, a future study integrating SNP genotyping, studying IGF1 mRNA expression profiles, serum IGF1 levels and phenotypic appraisal of birds with different genotypes is recommended to decipher and confirm possible mechanisms underlying the current observations.

Both IGF1SNP3T>C and IGF2SNP2 were synonymous mutations and did not alter the primary structure of gIGF1.This probably explains why these SNPs had no effect on early growth traits in local guinea fowls. IGF2SNP1 which is located in an intronic region did not affect body weight or growth traits during the study as is common to most SNPs within introns as indicated by Schmid *et al*. (2005).

The CT genotypes present at 56,018,958 bp and 56,018,966 bp locations within the 5'UTR of *gIGF1* were associated significantly with high body weights from the fourth week and beyond. Similarly, the genotypes TT and GG were significantly associated with higher

body weights beyond the fourth week compared to TA and GA genotypes at 56,069,114 bp and 56,069,127 bp within the 3'UTR. Therefore, it could be predicted that the haplogroups with the genotypes CT for SNPs within 5'UTR and TT, GG for SNPs within 3'UTR including H1H7, H1H4 would record greater body weights. Indeed the haplogroup HIH7 was the haplogroup with the highest body weights from the fourth week and for overall growth rate. Haplogroup H1H4 recorded mid-range body weights. It can also be predicted that the haplogroup containing TT, AT, AG at polymorphic loci from 5' to 3' should record the lowest body weights. Confirming this, the haplotype H4H5 recorded the lowest body weights beyond the fourth week. Therefore, evidence from the statistical associations of genotypes at the individual polymorphic loci within 5'UTR, 3'UTR and haplogroups provide preliminary evidence for SNPs in *gIGF1* significantly associated with body weights beyond the fourth week in guinea fowls. However, influence of haplogroups on body weight traits appear to be more complex than when effect of polymorphisms is considered on individual SNP basis probably due to integrated effects of the SNPs and influence of other cis-regulatory elements.

Currently there is no report on local breeds of guinea fowls established through selective mating and maintained with accurate recording schemes that could be used during the current study to determine SNP-trait associations with known pedigree data. Therefore, it is recommended that such breeding programmes aiming at establishing divergent guinea fowl lines for growth rates be initiated to enable evaluation of SNP-trait associations with pedigree data. Until the establishment of such breeds, the current study provides preliminary evidence for SNPs of which relevance can be further explored as candidate SNPs for marker assisted selection. With availability of whole genome sequence which lay

the foundation for development of high density SNP panels in the future, the novel SNPs in *gIGF1*and their associations identified may facilitate representation of local breeds in SNP chips in the future.

Conclusion

Either IGF1SNP1C>T or IGF1SNP2C>T or both present on chromosomal locations 56,018,958 bp and 56,018,966 bp, respectively, are likely to influence the body weight traits and growth rates during early growth of local guinea fowls originating from Northern Ghana. Similarly, either SNPs IGF1SNP4T>A or IGF1SNP5G>A or both are also likely to influence body weight traits and growth rates by influencing translation efficiency or mRNA stability in indigenous guinea fowls. While present observations provide preliminary evidence for SNP markers associated with early growth traits, further research should aim at establishing diverse lines of guinea fowls from selective breeding to determine the genotype-early growth trait associations in experimental crosses with pedigree data available and to examine the influence of the SNPs on mRNA and serum IGF1 levels.

CHAPTER 8

GENERAL DISCUSSION

8. GENERAL DISCUSSION

Faster growth is one of the quantitative traits most desired by poultry farmers. Early growth is important in poultry breeding programmes aiming at improving overall growth rate and slaughter weight. Body weights and growth rates during early growth period are often used as selection criteria in breeding programmes aiming at improving overall growth rate in poultry (ALBC, 2007). Body weight and overall growth rate are influenced by genetic factors, environmental factors and their interactions (Ayorinde, 2007). Genes coding for the complex network of protein involved in regulation of growth are among the major genetic factors regulating early growth in poultry (Kim, 2010). Major environmental factors that influence early growth in poultry include feeding, husbandry practices and health management. An array of pre-incubation and incubation factors (Tona *et al*., 2004) including breeder age (Peebles *et al*., 1999), breeder nutrition (Heier *et al*., 2002), egg pre-incubation storage conditions and storage time (Decuypere and Bruggeman, 2007) have dominant effects on body weights and growth rates particularly during the post-hatch period.

Results observed during the performance appraisal of young guinea fowls from the three populations of Northern Ghana (TPNG) indeed can be largely attributed to influence of these environmental and genetic factors and their complex interactions. Although, there were very limited variations among the TPNG at some weeks prior to the fourth week, local guinea fowls from the TPNG did not vary significantly in body weight and growth rate beyond the fourth week. Therefore, the local guinea fowl population of Northern Ghana may be viewed as a uniform population in terms of body weight traits and growth rates.

Significant variations in body weight and growth rates were however evident within the regional populations from the second week ($p < 0.05$). These within population and between subpopulation phenotypic variations were pronounced after the sixth week ($p < 0.001$). As evident by the growth curves for the subpopulations per each regional population (Chapter 4: Figures 12, 13 and 14) subpopulations N4, E3 and W2 were superior to other subpopulations in growth rates. As the birds were incubated and raised under similar experimental conditions, these variations may be attributed to a combination of pre-incubation and genetic factors.

During the first four weeks the experimental flock at CSIR-ARI (ARI flock) recorded higher body weights than TPNG probably due to better breeder stock management in their parents compared to the TPNG. However, from the sixth week there were no significant differences between ARI flock and the TPNG. Local guinea fowls from some subpopulations also grew faster i.e. N4 (Chapter 4: Figure 13), E3 (Chapter 4: Figure 12) and W2 (Chapter 4: Figure 14) and weighed heavier than the ARI flock beyond the sixth week. Better growth in keets of these subpopulations compared to keets from ARI flock irrespective of better managed breeder flock from which keets were hatched suggest more involvement of genetic factors rather than pre-incubation factors.

As these statistical effects became prominent with advancement of age, influence of these factors on slaughter weight may be significant and need to be investigated by extending the study period during future research. Most importantly, these differences residing within the regional populations can be utilized to develop faster growing strains of guinea fowls for growth rate and body weight traits through selective breeding utilizing genetic resources of indigenous guinea fowls.

Although, ARI flock was comparable to TPNG and most of the subpopulations in terms of body weights and growth rates they were outstanding in terms of survivability during the first eight weeks (Chapter 4: Figures 15 and 16) recording a cumulative keet mortality of only 7.84 % against a range of 63.64% (N2) to 100% (E1; Table 7) among the subpopulations. Due to the persistent reports of high cumulative mortalities during the first eight weeks in local guinea fowls from NG (Avornyo *et al*., 2016) and across tropical Africa (Moreki and Radikara, 2013) several workers have proposed the importance of post-hatch brooding under intensive management as an intervention to minimize keet mortalities (Dei *et al*., 2009; Avornyo *et al*., 2015; Mohammed and Dei, 2017). However, despite some farmers in NG adopting post-brooding management they still report high keet mortalities (Avornyo *et al*., 2014). As all the groups for different subpopulations and ARI flock were raised under the same post hatch brooding management with stringent biosafety measures, this difference in survivability of the ARI flock and the other populations provide some evidence for the importance of breeder stock management on keet survivability rather than early growth. High rate of keet mortality within the first 8 weeks has been ranked as the single overriding challenge faced by the guinea fowl farmers persistently (Avornyo *et al*., 2016). Although evaluating the factors affecting keet survivability was not among the objectives of this study observations made regarding disparities in survivability are of great importance to rural farmers of NG. Integrating the benefits of post-brooding management with breeder stock management may be the solution for the overriding challenge of keet mortality in NG.

Similarly, although examining the factors influencing the body weight, growth rate and survivability during the early post-hatch period (0-4 weeks post hatch) were not among the

objectives of this study, the observations provide insights into the importance of multiple "pre-incubation factors" that influence body weights, growth rates and survivability in guinea fowls. However, literature on these pre-incubation factors are extremely limited for local guinea fowls. Therefore, future research is recommended to ascertain the influence of such pre-incubation factors including feeding and nutrition of breeders, length of egg storage and storage conditions on body weights, growth rates and survivability of guinea fowl keets through both on station trials and on-farm experimentation. Future research should also be extended to develop guidelines for breeder stock management, collection of eggs and storage of eggs to ensure quality of keets hatched.

Based on the observed variations within the populations in terms of body weight and growth rates during early growth, future breeding programmes should aim at utilizing these variations to establish faster growing guinea fowl breeds from local varieties. Implementation of such selection programmes can be complemented by Marker Assisted Selection (MAS). MAS demands availability of informative markers identified from QTL mapping. Identification of such QTLs traditionally demands large populations of animals with extensively pedigreed data. Recently QTL mapping has been achieved with relatively a few marker informative matings of crosses from outbred populations (FAO, 2007b). However, there is no information on specialized guinea fowl breeds or populations established from outbred populations with pedigreed data in Ghana. Application of candidate gene approach has been proposed as a stepping stone for MAS for outbred populations as those from the three main populations sampled during the current study (FAO, 2007b).

In this study, the Identification of six SNPs within the exonic regions of IGF1 (*gIGF1*) and two SNPs in IGF2 gene (*gIGF2*) in guinea fowls opens the prospects of utilizing these SNPs to complement breeding programmes aiming at improving local breeds of guinea fowls. Further, AMOVA results also indicated that 99% of variations arising from the SNPs within functional regions of *gIGF1* reside within the populations. In this study variations within and among populations by region were only assessed based on the SNPs within exonic regions of IGF1 and IGF2. Therefore, it is likely there are genetic factors contributing these differences in growth between the subpopulations.

Considering the importance of SNPs within exons of candidate genes in influencing a QTL (Schmid *et al*., 2005), the identification of six novel SNPs within the functional regions of *gIGF1* and *gIGF2* is an important first step. None of the two SNPs identified within the *gIGF2* including IGF2SNP1 and IGF2SNP2 located at 13,955,730 bp and 13,956,496 bp on the sixth chromosome had any statistical effects on body weight traits and growth rates during early growth in local guinea fowls and hence are likely to be less useful as candidate SNPs for early growth in guinea fowls. In contrast, the two SNPs found within the 5'UTR and those found within 3'UTR had significant effects on body weight traits and growth rates from the second and fourth week, respectively. Such "statistical associations" may not necessarily imply a significant "functional association" as there may be other SNPs with functional associations due to an effect known as the Baevis effect (Goddard and Hayes, 2009). However, a critical review and analysis of the pivotal role of IGF1 in growth regulation in poultry, importance of the 5'UTR and 3'UTR in regulation of gene expression suggest that the polymorphisms found in the 5'UTRand the 3'UTR indeed are likely to act by influencing the expression of *gIGF1* at translation level. Unlike the many non-

synonymous mutations and SNPs within introns which are in LD that often results in false positive associations with causal sites in GWAS, the four SNPs that had statistical associations are likely to have a functional association with early growth in guinea fowls. However, available information suggests dominant roles for 5'UTR in regulating gene expression at translation level, an effect dominant with growth factors (Pesole *et al*., 2002; Gebauer and Hentze, 2004; Arajuo *et al*., 2012). Similarly, 3' UTR has been associated with regulating protein expression also at translation level by influencing mRNA localization, recycling of translation apparatus and importantly by influencing mRNA decay (Brown and Sachs, 1998; Jansen 2001; Mignone *et al*., 2002). The SNPs identified within the 5'UTR and 3'UTR are likely to be causal sites for important quantitative traits of body weight and growth rates in guinea fowls. This study thus provides the first line of evidence of novel SNPs that are associated with early growth traits in local guinea fowls from NG. Associations of these SNPs should be further analysed within populations of local guinea fowls in structured breeding programmes parallel to mRNA profiling and measuring of serum IGF1 levels to facilitate deciphering of specific molecular mechanisms and realization of fullest potential of MAS, GS and genetic resources of this underutilized indigenous species to bridge the disparities of protein intake of the people of Northern Ghana.

CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS

9.1. Conclusions

On the basis of the findings from this study, it can be concluded that there exist considerable variation in juvenile body weight traits and growth rates among subpopulations of local guinea fowls from the three main populations of Northern Ghana.

Due to limited variation evident from the presence of only one synonymous Single Nucleotide Polymorphism in protein coding sequences in the Insulin-like Growth Factor 1 gene in guinea fowls (*gIGF1*) from NG, the IGF1 proteins synthesised by the local guinea fowls across NG are likely to be similar in primary, secondary and tertiary structures. The genotypes arising from this synonymous mutation in protein coding segments of IGF1 gene in guinea fowl (*gIGF1*) had no effects on juvenile body weight traits and growth rates among the local guinea fowls from the TPNG.

Unlike the amino acid coding sequences in the exons of *gIGF1*, there was greater variation within the 5' and 3' untranslated regions of IGF1 gene in local guinea fowls from NG evident by the presence of five SNPs in these regions of *gIGF1*.

The different genotypes arising from Single Nucleotide Polymorphisms (SNPs) present within the 5'and 3' untranslated regions were statistically associated with juvenile body weight traits and growth rates and may be useful in Marker Assisted Selection in improving juvenile growth rates in indigenous guinea fowls from NG.

There was also limited variation based on the SNPs within genomic sequences spanning the third and fourth protein coding exons of Insulin-like Growth Factor 2 gene (*IGF2*) among local guinea fowls from the TPNG but the genotypes arising from these SNPs were not associated with juvenile body weight traits and growth rates.

9.2. Recommendations

Pre-incubation factors such as maternal diet, maternal nutrition, pre-incubation storage time and conditions appear to influence early post-hatch growth. It is therefore, recommended that future research be conducted to bridge the knowledge gap on these factors influencing early post-hatch growth in guinea fowls.

Farmers should be sensitized on the influence of breeder stock management on survivability of keets. Future research should aim at developing breeder stock management strategies that can be adopted by farmers in NG. Stakeholders should train guinea fowl farmers in NG and other tropical African countries on integrating good practices for breeder stock management and post-hatch brooding to reduce the burden of high keet mortality in the sub-region.

Based on the observations on differences in body weight traits and growth rate traits among the subpopulations, it is recommended that future research explores the differences in body weight traits and growth rates among the subpopulations within the larger populations of local guinea fowls throughout the entire growth period up to slaughter stage.

Considering the definite adaptive advantages of indigenous breeds for the local farmers and the preference for faster growing guinea fowls, future breeding programmes can further explore the observed differences in body weights and growth rates residing within the populations from the three populations of Northern Ghana to establish faster growing guinea fowl lines as a sustainable alternative to introduction of exotic guinea fowls or cross-breeding. Therefore, the solutions for two main challenges of guinea fowl industry including high level of keet mortality and slow growth in Northern Ghana are likely to be

solved by initiating structured breeding programmes during which local guinea fowls are selectively bred by phenotypic selection adhering to good breeder stock management practices with integrated improvements in management, feeding and health management.

Based on the observed statistical associations of the SNPs within the 5'UTR and 3'UTR of *gIGF1* in local guinea fowls, usefulness of these SNPs in breeding programmes should be further explored by evaluating SNP-trait associations within informative matings starting from a larger population of outbred local guinea fowls in Northern Ghana.

Subsequent to such informative matings, structured breeding programmes aimed at improving growth rate with high survivability should be initiated using local guinea fowls during which utility of the identified SNPs in MAS should be explored to complement and accelerate expected genetic gain in body weight and growth rate traits in indigenous guinea fowls. Furthermore, evaluation of associations between these SNPs and traits also should be extended beyond the eleventh week up to slaughter stage to study the influence of these SNPs during the entire growth period and slaughter traits.

The functions of 5' and 3'UTRs in genes coding for proteins involved with cell cycle regulations suggest a potential role of these polymorphisms in regulating IGF1 gene expression at the translational level. It is thus recommended that this hypothesis should be examined by implementing a cross-sectional study during which concentrations of serum IGF1 and mRNA variants are monitored during the entire growth period for different genotypes to establish ultimate molecular evidence for the observed statistical associations in the study.

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APPENDICES

Appendix 1. Number of guinea fowl eggs collected from the three populations of Northern Ghana, CSIR-ARI flock, their fertility and hatchability

Population	Subpop.	Sample Locations	No. of eggs collected	No. of eggs set	Fertility (%)	No. of keets Hatched	Hatchability (%)
Upper East Region	E1	Kayoro	47	47	68.09	16	50
		Sub total	**47**	**47**	**68.09**	**16**	**50**
	E2	Sandema	333	300	70	60	28.57
		Bongo	149	140	69.29	24	24.74
		Bolgatanga	304	296	59.12	34	19.43
		Nangodi	360	349	63.9	35	15.7
		Sub total	**1146**	**1085**	**64.98**	**153**	**21.70**
	E3	Garu	231	199	55.78	9	8.11
		Pusiga	284	269	53.9	12	8.28
		Sub total	**515**	**468**	**54.7**	**21**	**8.20**
	E4	Zebilla	271	263	63.5	61	36.53
		Sub total	**271**	**263**	**63.5**	**61**	**36.53**
		Total for UER	**1979**	**1863**	**62.27**	**251**	**21.64**

Appendix 1(Contd.). Number of guinea fowl eggs collected from the three populations of Northern Ghana, CSIR-ARI flock, their fertility and hatchability

Population	Subpop.	Sample Locations	No. of eggs collected	No. of eggs set	Percentage Fertility	No. of keets Hatched	Percentage Hatchability
Former Northern Region(FNR)	N1	Yapei	127	97	92.78	54	60
		Bole	74	50	40	0	0
		Damongo	187	177	77.4	29	21.17
		Sub total	**388**	**324**	**76.23**	**83**	**33.6**
	N2	Daboya	179	150	47.33	18	25.35
		Tolon	199	170	82.35	8	5.71
		Kumbungu	199	170	82.35	52	37.14
		Nanton	40	39	76.92	10	33.33
		Sub total	**617**	**529**	**72.02**	**88**	**23.1**
	N3	Bunkpurugu	68	56	58.93	6	18.18
		Nalerigu	145	127	81.89	39	37.50
		Sub total	**213**	**183**	**74.86**	**45**	**32.85**
	N4	Wulensi	40	40	77.5	10	32.26
		Bimbila	65	60	75	17	33.78
		Sub total	**105**	**100**	**76**	**27**	**35.53**
		Total for FNR	**1323**	**1136**	**74.03**	**243**	**28.89**

Appendix 1 (Contd.). Number of guinea fowl eggs collected from the three populations of Northern Ghana, CSIR-ARI flock, their fertility and hatchability

Population	Subpop.	Sample Locations	No. of eggs collected	No. of eggs set	Percentage Fertility	No. of keets Hatched	Percentage Hatchability
Upper West Region	W1	Sambo	112	110	89.09	52	53.06
		We	60	50	70	17	48.57
		Wa	174	160	59.38	30	31.58
		Kaleo	90	89	80.9	25	34.72
		Sub total	**436**	**409**	**73.35**	**124**	**41.33**
	W2	Tumu	99	74	100	36	48.65
		Sub total	**99**	**74**	**100**	**36**	**48.65**
	W3	Chepuri	64	57	94.74	30	55.56
		Kokoligu	98	89	55.06	8	16.33
		Jirapa	217	207	74.4	25	16.23
		Babille	418	355	72.11	56	21.88
		Lawra	144	132	83.33	35	31.82
		Hamille	101	101	29.7	1	3.33
		Nandom	198	198	21.21	8	19.05
		Sub total	**1240**	**1139**	**61.02**	**163**	**23.45**
		Total for UWR	**1775**	**1622**	**65.91**	**323**	**19.91**
ARI			**163**	**161**	**65.83**	**51**	**48.11**

Appendix 2.Genomic sequences with SNPs of target exons of IGF1 gene in guinea fowls (*gIGF1*) generated during the current study

Target region in guinea fowl in *gIGF1*	Genomic sequence (5'to 3')‡	Origin
Exon 1	TCTCTAAATCCCTCTTCTGTTTGCTAAATCTCACTGTCACTGCTAAAATCA GAGCAGATAGAGCCTGCGCAATGGAATAAAGTCCTCAATATTGAAATGT GACATTGCTCTCAACATCTCACATCTCTCTGGATTTCTTTTTTCTCATCAT TACTGCTAACAAATTCATTTCCAGACTTTGCACTTTTAAGAAGCAATGGA AAAAATCAACAGTCTTTCAACACAATTAGTTAAGTGCTGCTTTTGTGATT TCTTGAAG	Local guinea fowls from Nalerigu, former Northern Region, Ghana with SNP variants T,T at locations 56018958, 56018966 bp on chromosome 1
	TCTCTAAATCCCTCTTCTGTTTGCTAAATCTCACTGTCACTGCTAAAATCA GAGCAGATAGAGCCTGCGCAATGGAATAAAGTCCTCAATATTGAAACGT GACATCGCTCTCAACATCTCACATCTCTCTGGATTTCTTTTTTCTCATCAT TACTGCTAACAAATTCATTTCCAGACTTTGCACTTTTAAGAAGCAATGGA AAAAATCAACAGTCTTTCAACACAATTAGTTAAGTGCTGCTTTTGTGATT TCTTGAAG	Local guinea fowls from Sandema, Upper East Region, Ghana with SNP variant C, C at locations 56018958, 56018966 of chromosome 1

Appendix 2 (Contd.).Genomic sequences with SNPs of target exons of IGF1 gene in guinea fowls (*gIGF1*) generated during the current study

Exon 3 Second Protein coding exon	GTGAAGATGCACACTGTGTCCTACATTCATTTCTTCTACCTTGGCCTGTG TTTGCTTACCTTAACCAGTTCGGCTGCTGCTGGCCCAGAAACACTGTGTG GTGCTGAGCTGGTTGATGCTCTTCAGTTTGTATGCGGAGACAGAGGCTTC TACTTCA	Local guinea fowl from Tumu, Upper West Region, Ghana with SNP variant C at 56,023,560 bp location on chromosome 1
	GTGAAGATGCACACTGTGTCCTACATTCATTTCTTCTACCTTGGCCTGTG TTTGCTTACCTTAACCAGTTCGGCTGCTGCTGGCCCAGAAACACTGTGTG GTGCTGAGCTGGTTGATGCTCTTCAGTTTGTATGCGGAGACAGAGGCTTT TACTTCA	Exotic guinea fowl from a domestic French breed with SNP variant T at 56,023,560 bp location on chromosome 1

Appendix 2 (Contd.).Genomic sequences with SNPs of target exons of IGF1 gene in guinea fowls (*gIGF1*) generated during the current study

Exon 5 (Fourth protein coding exon)	GAAGTGCATTTGAAGAATACAAGTAGAGGGAACACAGGAAACAGAAAC TACAGAATGTAAGATCATGCCATCCACAAGAATGAAGAATGAATGTGCC ATCTGCAGGATACTTTGCTGTAAATAAATTATTTGTTAAACATTGGAAGA CTA	Local guinea fowl from Bolgatanga, Upper East Region, Ghana with SNP variants T, G and G at bp locations 56069114, 56069127, 56,069,164 on chromosome 1
	GAAGTGCATTTGAAGAATACAAGTAGAGGGAACACAGGAAACAGAAAC TACAGAATGTAAGATCATGCCATCCACAAGAATGAAGAATGAAAGTGCC ATCTGCAAGATACTTTGCTGTAAATAAATTATTTGTTAAACATTGGAAGA CTA	Local guinea fowl from Sandema, Upper East Region, Ghana with SNP variants A, A and G at bp locations 56069114, 56069127, 56,069,164 on chromosome 1
	GAAGTGCATTTGAAGAATACAAGTAGAGGGAACACAGGAAACAGAAAC TACAGAATGTAAGATCATGCCATCCACAAGAATGAAGAATGAATGTGCC ATCTGCAGGATACTTTGCTGTAAATAAATTATTTGTTAAACATTAGAAGA CTA	Local guinea fowl from Bimbilla, former Northern Region, Ghana with SNP variants T, G and A at bp locations 56069114, 56069127, 56,069,164 on chromosome 1

Appendix 3. Sequences of amplified *IGF2* genomic regions in guinea fowls during the current study

Target region in guinea fowl	Genomic sequence (5'to 3')	Origin
Exon 4	GGGTGACCAGCAATGGGTAGAGGAAACCCCAGGGCAGCGTTGGTAAC CCACCTCGTGTTGACACTGCGTTGCTCTCCCTTCCTCAGGAGAGCTTCC AGAAGCCATCTCACGCCAAGTACTCCAAGTACAACGTGTGGCAGAAGA AGAGCTCGCAGCGGCTGCAGCGGGAGGTGCCTGGCATCCTGCGCGCCC GCCGGTACCGGTGGCAGGCGGAGGGGCTGCAAGCAGCCGAGGAAGCC AGGGCGATGCATCGTCCCCTCATTTCCTTGCCCAGCCAGCGGCCCCCGG CGCCGCGGGCATCCCCTGAAGCGACCGGCCCCCAGGAATGAACTGTGA CCGGCCAGCTCGATTTGTGATCTCCTGGGGAGAGACTGGCGAGACTCG GCCCCCCTGAGCCCCTCCGTCCCCGAGCCAAGGAGCGGGGCGGCAGGC ACCATCACGCCGCTCCGG	Male guinea fowls of local breeds from Sandema, Upper East region, Ghana with homozygous genotypes GG at locations 13956496 bp of chromosome 6

Appendix 3 (Contd.). Sequences of amplified *IGF2* genomic regions in guinea fowls generated during the current study

Exon 4	GGGTGACCAGCAATGGGTAGAGGAAACCCCAGGGCAGCGTTGGTAAC CCACCTCGTGTTGACACTGCGTTGCTCTCCCTTCCTCAGGAGAGCTTCC AGAAGCCATCTCACGCCAAGTACTCCAAGTACAACGTGTGGCAGAAGA AGAGCTCACAGCGGCTGCAGCGGGAGGTGCCTGGCATCCTGCGCGCCC GCCGGTACCGGTGGCAGGCGGAGGGGCTGCAAGCAGCCGAGGAAGCC AGGGCGATGCATCGTCCCCTCATTTCCTTGCCCAGCCAGCGGCCCCCGG CGCCGCGGGCATCCCCTGAAGCGACCGGCCCCCAGGAATGAACTGTGA CCGGCCAGCTCGATTTGTGATCTCCTGGGGAGAGACTGGCGAGACTCG GCCCCCCTGAGCCCCTCCGTCCCCGAGCCAAGGAGCGGGGCGGCAGGC ACCATCACGCCGCTCCGG	Local guinea fowls from Lawra, Upper West Region, Ghana with Homozygous genotype AA at locations 13956496 bp of chromosome 6

Appendix 3 (Contd.). Sequences of amplified *IGF2* genomic regions in guinea fowls generated during the current study

Exon 3 and part of adjacent intron	GGTAGACCAGTGGGACGAAATAACAGGAGGATCAACCGCGGCATCGT GGAGGAGTGCTGCTTTCGGAGCTGCGACCTGGCTCTGCTGGAAACCTA CTGCGCCAAGTCCGTCAAATCAGAGCGTGACCTCTCCGCCACCTCCCTC GCGGGCCTCCCAGCCCTCAACAAGGTAGGGCTGCACTGGGGCTGCTAG CTCCCTGAAGCAAGAAAGATGGGAAAAGGGAGGCCATGGGTGGGGGG GGACTGCTGCCGGCTCTAAGGTCTGCTGCTTCTGCAGCCAAGGCAGCAT GCAGAGCCTTGAAGTGTTTATAGCTGTTGAGGAGTCAAAGAAATTGCA GAAAACAAACTTATAACCACCAAATCATAGAACGGTTTGGGTTGGAAG AGCCCCCTAGTGATTATCTAGTCCAGGGACCTTTAAAGATCATCTAGTC CAGCCCTCTGCCATGGGCAGGGACACATCCCACTAGGTCAGGTTGCCC AAAGG	Local male guinea fowl from Lawra, Upper West region with no insertion adjacent to 13955730 bp location of reference guinea fowl sequence of chromosome 6

Appendix 3 (Contd.). Sequences of amplified *IGF2* genomic regions in guinea fowls generated and submitted to GenBank during the current study

Exon 3 and part of adjacent intron	GGTAGACCAGTGGGACGAAATAACAGGAGGATCAACCGCGGCATCGT GGAGGAGTGCTGCTTTCGGAGCTGCGACCTGGCTCTGCTGGAAACCTA CTGCGCCAAGTCCGTCAAATCAGAGCGTGACCTCTCCGCCACCTCCCTC GCGGGCCTCCCAGCCCTCAACAAGGTAGGGCTGCACTGGGGCTGCTAG CTCCCTGAAGCAAGAAAGATGGGAAAAGGGAGGCCATGGGTGGGGGG GGGACTGCTGCCGGCTCTAAGGTCTGCTGCTTCTGCAGCCAAGGCAGC ATGCAGAGCCTTGAAGTGTTTATAGCTGTTGAGGAGTCAAAGAAATTG CAGAAAACAAACTTATAACCACCAAATCATAGAACGGTTTGGGTTGGA AGAGCCCCCTAGTGATTATCTAGTCCAGGGACCTTTAAAGATCATCTAG TCCAGCCCTCTGCCATGGGCAGGGACACATCCCACTAGGTCAGGTTGC CCAAAGG	Local female guinea fowl from Kumbunugu, former Northern Region, Ghana with G insertion adjacent to 13955730 bp location of reference guinea fowl sequence of chromosome 6